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BIOMARKERS OF MAJOR DEPRESSIVE DISORDER

A study of the interaction of genetic, neuroimaging and endocrine factors, and the effects of childhood adversity, in major depressive disorder

Sendi, Shahbaz

Awarding institution:
King's College London

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BIOMARKERS OF MAJOR DEPRESSIVE DISORDER

A study of the interaction of genetic, neuroimaging and endocrine factors, and the effects of childhood adversity, in major depressive disorder

Shahbaz Sendi/1145361/Ph.D.

2012-2016

Happy, that is all I wanted to be in my life

To my loved ones

Abstract

My thesis consisted of two studies. The first study was a part of a wider study; within this, we investigated the modulation of amygdala structure by the val66met BDNF (Brain Derived Neurotrophic Factor) polymorphism. Structural Magnetic Resonance Imaging (MRI) scans were obtained at 1.5 T in 87 Major Depressive Disorder (MDD) patients and 74 age, gender, and verbal IQ matched healthy controls. We used Freesurfer version 5.1.0 to examine the grey matter amygdala volume.

In the second study, we investigated neuroendocrine abnormalities-- Hypothalamus-Pituitary-Adrenal Axis (HPA) axis changes-- in MDD and their relation to early life stress (ELS). In total 112 subjects took part, consisting of MDD patients with (n=28) and without (n=15) a history of ELS and healthy controls with (n=26) and without (n=43) a history of ELS. The cortisol awakening response (CAR) was used as an index of HPA axis activity.

In both studies, the data were analyzed using Statistical Package for Social Science (SPSS version 22).

In the first study, we did not find any modulatory effect of the val66met polymorphism on the grey matter of right and left amygdala volumes. In the second study, we showed that the CAR was most elevated in those who were both depressed and had a history of ELS, which supports the argument that the effects of early life stress and MDD on the HPA axis may be additive.

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Abbreviations

Abbreviation	Meaning
5HTTLPR	Serotonin-Transporter-linked Polymorphic Region
ACAT	Acyl CoA cholesterol Transferase
ACC	Anterior Cingulate Cortex
ACTH	Adrenocorticotrophic hormone
ADNI	Alzheimer's Disease Neuroimaging Initiative
AUC	Area Under the Curve
AUCg	Area Under the Curve with respect to the ground
AUCi	Area Under the Curve with respect to the increase
BDI	Beck Depression Inventory
BDNF	Brain Derived Neurotrophic Factor
BMI	Body Mass Index
CAR	Cortisol Awakening Response
CBG	Corticosteroid Binding Globulin
COMT	Catechol-O-Methyltransferase
CRH	Corticotropin-Releasing Hormone
CSF	CerebroSpinal Fluid
CTQ	Childhood Trauma Questionnaire (CTQ)
DLPFC	Dorso Lateral Prefrontal Cortex
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSM	Diagnostic and Statistical Manual of Mental Disorders
ELS	Early Life Stress
EW	Emotional Wellbeing
FDG-PET	Functional (18F)-fluoroDeoxyGlucose Positron Emission Tomography
FE	Fatigue/Energy
fMRI	Functional Magnetic Resonance Imaging
GAD	General Anxiety Disorder
GFP	Green Fluorescent Protein
GH	General Health
HAMD	Hamilton Depression Rating Scale
HMO	Health Maintenance Organization
HPA	Hypothalamus-Pituitary-Adrenal
HSL	Hormone Sensitive Lipase
ICD	International Classification of Diseases
ICD	International Classification of Diseases Manual
IDS	Inventory of Depressive Symptomatology
IES	Impact of Events Scale
LDL	Low-Density Lipoproteins
LTE	List of Threatening Events
MDD	Major Depressive Disorder
Met	Methionine
MOS	Medical Outcome Survey
MRI	Magnetic Resonance Imaging
NAA	N-Acetyl Aspartate
OCD	Obsessive Compulsive Disorder
OCPD	Obsessive Compulsive Personality Disorder
OSS-3	OSLO Social Support Scale
PET	Positron Emission Tomography
PF	Physical Functioning
POMC	Proopiomelanocortin

PSQI	Pittsburgh Sleep Quality Inventory
PSS	Perceived Stress Scale
PTSD	Post Traumatic Stress Disorder
PVN	Paraventricular Nucleus of hypothalamus
RDC	Research Diagnostic Criteria
RL-E	Role Limitation due to Emotional problem
RL-PH	Role Limitation due to Physical Health
SCAN	Schedule for the Clinical Assessment in Neuropsychiatry
SCN	Supra Chiasmatic Nucleus
SF	Social Functioning
SF-36	Survey Short Form 36
SPSS	Statistical Package for Social Sciences
SSRI	Selective Serotonin Reuptake Inhibitor
TCA	Tricyclic Antidepressant
TR-FIA	Time-Resolved Fluoroimmunoassay
Val	Valine
VBM	Voxel Based Morphometry
vmPFC	Ventromedial Prefrontal Cortex
WSAS	Work and Social Adjustment Scale

Chapter 1 Introduction

1.1 Major Depressive Disorder

1.1.1 Overview

Depression is the most common of the affective disorders. It is a debilitating worldwide mental illness, which is included in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition DSM-5 (American Psychiatric Association, 2013) and in the International Classification of Diseases Manual 10th Edition (ICD-10).

1.1.2 Epidemiology of major depressive disorder

It has been estimated that depression will be the second major cause of disability after ischemic heart disease by 2020 (Murray et al., 1997). The point prevalence of depression among those 16-74 years of age was 2.6% in the UK, in the year 2000 (Singleton et al., 2001). Kessler et al. (2005) argued that the lifetime prevalence of depression is 16.6%, which significantly changes when considering age group. While among the 30-44 years old, the rate is at its highest at 19.8%, it reaches its lowest at 10.8% in adults over 60 years old (Kessler et al., 2005). Women are 50% more likely to be recognized with an affective disorder during their life compared with men; thus, depression affects about 1 in 6 women and 1 in 10 men (Kessler et al., 2005; Craighead et al., 2014). Reports suggest that the prevalence of depression is not equal in all countries. This may show the impact of a number of issues on the prevalence of depression, such as clinical diagnostic problems and the influence of countries cultures. While 12 month prevalence of major depressive disorder (MDD) has been measured at 5-8% in Europe (Andlin-Soboki et al., 2004), Kessler et al. (2003) estimated it to be 6.6% in the USA. The highest rate was seen in Morocco at 26.5% (Kadri et al., 2010) and the lowest

rate was observed in China at 3.6% (Lee et al., 2009) and Japan at 3-7% (Kawakami et al., 2007).

1.1.3 Risk factors for major depressive disorder

The main risk factors for the development of depression are female gender, early life stress (Nemeroff et al., 2003), family history of mood disorders, former anxiety disorders, substance abuse and life events (Neigh et al., 2013).

1.1.4 Clinical assessment of major depressive disorder

Patients who are suspected of depression are normally recognized with clinical interviews. In regards to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 1994) depression is defined as a mental illness in which a person experiences a core feature of persistent low mood and/or anhedonia. People who have five or more of these following symptoms over a two-week period are recognized as having MDD: depressed mood for most of the day, anhedonia, significant weight loss, insomnia or hypersomnia, psychomotor agitation or retardation, loss of energy or fatigue, feeling guilt, poor concentration or having no ability to make a decision, and recurrent thoughts of death or suicide. At least one of these symptoms must be depressed mood or loss of pleasure in activities (DSM-IV, 1994). Social or occupational aspects of a person's life must be impaired, and there must not be any relation to the physiological effects of substances or a medical condition (DSM-IV, 1994). Similarly, in the International Classification of Disease, Tenth Revision (ICD-10, WHO, 1992), depressed mood, anhedonia and reduced energy are the three core symptoms, two of which should be seen to diagnose depression. ICD-10 (1992) criteria include additional symptoms such as sleep interruption, poor concentration or indecisiveness, low self-confidence, changed appetite, thoughts or acts of suicide, agitation, or slowing of movements, and guilt or self-blame.

1.1.5 Longitudinal course of major depressive disorder

A large number of studies have shown that the average age of the onset of depression is late adolescence or emerging adulthood, although this varies between studies (Hankin et al., 1999; Kessler et al., 2005).

MDD is a recurrent disorder (Craighead et al., 2014). Fergusson et al. (2002) have demonstrated that around 45% of adolescents who experience an episode of depression suffer a second episode before age 24. Moreover, Rohde et al. (2013) conducted a study in MDD patients about incidence, recurrence, gender, comorbidity, duration, and suicide attempts during childhood (age 5-12.9 years), using the data from 816 participants (56% female). They found that around 50% of adolescents had a second episode by the age of 30, reinforcing the recurrent nature of depression. Furthermore, a follow-up study with 406 patients, consisting of 186 patients with depressive disorder and 220 patients with bipolar disorder, found that there is an association between the number of previous episodes of depression and a higher risk of recurrence (Kessing et al., 2004).

In terms of treatment, a substantial minority of MDD patients do not respond to initial treatment, and are defined as having treatment resistant depression (TRD). In the short term, TRD patients have greater tendencies to be recurrent (Fekadu et al., 2007). Specifically, Rush et al. (2006) showed that more than 80% of those followed up after a response to treatment, relapse within a year. The rate of short-term remission in TRD is very low. One study found a rate of under 20% (Dunner et al., 2006).

Several studies have found that the duration of depressive episodes varies substantially between subjects (Craighead et al., 2014), and the average time of recovery varies from 5 to 13 months.

1.1.6 Treatment of major depressive disorder

Several forms of treatment are used for MDD (Craighead et al., 2014). While antidepressants and psychotherapies are the main treatments, stimulation treatments including transcranial magnetic stimulation, deep brain stimulation, vagus nerve stimulation and electroconvulsive therapy represent other more rarely used treatment options. In association with this, recently, Ketamine has been used for treatment of depression.

It is of note that combinations of treatments may be more efficacious than monotherapies; for example, Nemeroff et al. (2003) demonstrated that antidepressants and psychotherapies have broadly equivalent effectiveness, but remission rates are higher when using both treatments in combination.

There is little evidence suggesting preferential efficacy of individual antidepressants for the treatment of depression among all subjects (Anderson et al., 2012). There is no consistent evidence to show clinically relevant superiority of one class of antidepressant over another, while there are more than 7 classes of antidepressant (Lin et al., 2014).

Interestingly, one meta-analysis by Cipriani et al. (2009) assessed the effects of 12 new-generation antidepressants (Bupropion, Citalopram, Duloxetine, Escitalopram, Fluoxetine, Fluvoxamine, Milnacipran, Mirtazapine, Paroxetine, Reboxetine, Sertraline, and Venlafaxine) on MDD. The results suggested that, for the initial treatment of moderate to severe MDD in adults, Sertraline could be the best choice, balancing efficacy against adverse effects. In this study, Mirtazapine, Escitalopram, Venlafaxine, and Sertraline were considerably more efficacious than Duloxetine.

1.1.7 The global concern about major depressive disorder

Depression is a global mental health concern. The World Health Organization suggested that depression will be ranked as the second major cause of disease burden by 2020 (Menken et al., 2000).

1.1.7.1 The impact of major depressive disorder on personal lives

The personal and social influence of MDD is serious, in terms of personal suffering, disability and financial costs (Fekadu et al., 2009). Depression is associated with a high rate of suicide – 3.4% in the USA (Barlow, 2005), with a global rate of 1.3 to 4.4% (Walsh, 2009) – and disability. An increased risk of morbidity and mortality, including premature death from cardiovascular disease, stroke and diabetes, has been reported in some studies in association with depression (Barth et al., 2005; Egede et al., 2006; Loeb et al., 2012). Moreover, depression impairs the quality of relationships between people and within families (Arató et al., 1988; Rich et al., 1988). In line with this, MDD has a negative effect on general measures of patients' quality of life (Wells et al., 1989).

1.1.7.2 The economic burden and cost of major depressive disorder

In regards to the calculations of the costs of depression to societies, the economic burden of MDD is a large issue. Medical and psychological treatments of MDD exert a tremendous cost. Greenberg et al. (2003) has reported total costs of \$83.1 billion per year in 2000 in the USA, and Sobocki et al. (2006) estimated the cost for Europe at around €118 billion per year. In particular, patients with TRD accumulate higher medical and mental health care costs, due to the association of MDD with physical comorbidity and higher illness burdens, such as disability and suicide (Fekadu et al., 2008).

1.2 Early Life Stress

1.2.1 Overview

Child abuse is a serious social problem. It is a complex global phenomenon, which does not respect boundaries of class, nationality, race, religion, age, or educational level and can happen both publicly and privately (Carr et al., 2013; Juruena et al., 2013).

The consequences of child abuse may include biological, physiological, behavioural and cognitive dysfunction. It has been argued that child abuse could be a specific risk factor for psychiatric disorders such as unipolar depression. Rates of depression in adulthood are higher in those who have experienced child abuse. In a health maintenance organization (HMO) population, a dose–response association between the number of childhood abuse experiences and the presence of a recent-in the past year- depressive episode or lifetime chronic depression has been observed (Chapman et al., 2004). One previous study in this population found 4-fold increase in the risk of depression in individuals with multiple childhood adverse experiences (Felitti et al., 1998). In association with the increasing risk of depression in individuals experienced childhood abuse, Heim et al. (2010), argued that the underlying mechanism of the higher risk of depression in the subjects experienced early life stress could be due to persistent changes in Corticotropin-Releasing Hormone-mediated (CRH-mediated) stress responses. MDD is related to increase cerebrospinal fluid (CSF) concentrations of CRH, increased CRH immunoreactivity, increased CRH mRNA expression in the hypothalamic paraventricular nucleus of hypothalamus (PVN), and downregulation of CRH-1, but not CRH-2, receptors in the cerebral cortex (Nemeroff et al., 1999; Heim et al., 2010). A number of clinical studies suggested

that childhood abuse may contribute to CRH and Hypothalamus-Pituitary-Adrenal (HPA) axis changes observed in depressed patients (Heim et.al 2000; 2002).

The exact prevalence of child abuse is unclear for a variety of reasons, but estimates vary from 25% to 45% (Costello 1998; Heim et al., 2010).

Considering the definition of early childhood experience which is a broad concept, the four main types of early life stress include: emotional abuse, physical abuse, sexual abuse, and neglect (physical and emotional) (Jurueña et al., 2013). These are defined as follows:

1.2.2 Emotional

Verbal aggression that affects the welfare or the morale of a child or any conduct that humiliates, embarrasses, or threatens a child.

1.2.3 Physical

Physical aggression by someone older, with the risk of, or resulting in, injury.

1.2.4 Sexual

Any type of sexual contact or conduct between a child and someone older.

1.2.5 Neglect (physical, emotional)

Emotional neglect: failure of caretakers to provide for basic emotional and psychological needs such as love, motivation, and support.

Physical neglect: failure of caretakers to provide for basic physical needs such as feeding, a home, security, supervision, and health (Bernstein et al., 2003).

1.3 HPA axis

The HPA axis is one of the most crucial endocrine systems of the body and one of the main biological systems involved in the stress response.

1.3.1 Physiology of HPA axis

The endocrine system plays an important role in the integration and regulation of several functions of the body and is functionally integrated with the nervous and

circulatory system. In response to stress, CRH is released from the Paraventricular Nucleus of hypothalamus (PVN) into the portal venous circulation. CRH has the major role in stimulating the synthesis of Proopiomelanocortin (POMC) by the corticotroph cells, which is the precursor of Adrenocorticotrophic hormone (ACTH) subsequently released from the anterior pituitary cells. Therefore, three main roles of CRH involve the stimulating of POMC transcription, ACTH biogenesis and stimulating the release of ACTH. Increasing ACTH release activates glucocorticoid secretion from the adrenal cortex, which is the final part of the HPA axis. The adrenal cortex has the main role in the production of cortisol.

1.3.2 Cortisol

Cortisol has been hypothesised to have a crucial role in the onset and course of MDD, but there is no agreement as to what the exact role may be. Many unanswered questions have been raised during these years, and it remains unclear whether for example the evaluation of cortisol is necessary for MDD patients in routine clinical management or therapeutic processes. The optimal level of cortisol is not clear, which raises more doubts around reaching a conclusion about the exact role of cortisol (Herbert et al., 2013).

It has been assumed that the optimal range of cortisol is not the same for all individuals. Cortisol can be characterized in terms of absolute levels, or as the shape or amplitude of the cortisol rhythm over 24 hours; normal range values for each of these may vary depending on subjects' circumstances (Herbert et al., 2012).

Furthermore, it remains unclear to what extent the observed changes in cortisol levels – which will be explained later in my thesis – represent causes or effects of depression (Herbert et al., 2012).

In recent years, the cortisol awakening response (CAR) has been increasingly researched, and found to be reliable and useful measurement of HPA axis activity. Assessing the CAR is not an invasive test, and has been used for capturing information about stress reactivity in numerous studies (Pariante et al., 2008; Adam et al., 2010).

1.3.2.1 Cortisol synthesis

The adrenal cortex is made up of three zones: zona glomerulosa, zona fasciculata, and zona reticularis, comprising the outer, the middle and the inner zones. Their major endocrine products are not similar as each of them expresses specific complements of steroidogenic enzymes. The most actively steroidogenic zone is the zona fasciculata, which makes the glucocorticoid hormone, cortisol.

Captured low-density lipoproteins (LDL) particles, and cholesterol de novo made from acetate, are used to synthesize cortisol via a process consisting of several stages, within the large cells of zona fasciculata.

Within the foamy cytoplasm of the large cells, Acyl CoA cholesterol transferase (ACAT) esterifies free cholesterol. Cholesterol is stored in the lipid droplets, which can be turned back to free cholesterol by hormone sensitive lipase (HSL). ACTH can increase this process of turning back to free cholesterol.

A number of reactions within a steroidogenic pathway form cortisol. The first stage involves converting cholesterol to pregnenolone by the enzyme cytochrome P-450. After that, the enzyme 3 β -hydroxysteroid-dehydrogenase converts pregnenolone to progesterone. Following this stage, cortisol is formed by 11-hydroxylase after the production of 11-deoxycortisol.

1.3.2.2 Secretion, Effects and Receptors

Cortisol production is regulated by the activation of the HPA axis. Cortisol negatively feeds back on the hypothalamus and pituitary to control its set point.

Cortisol levels begin to rise during the early predawn and morning hours (between 01:00 a.m. and 04:00 a.m.) and reach a peak in the morning, then decrease during the daytime and evening hours to reach their lowest point at midnight. The free-hormone hypothesis (Mendel et al., 1989) proposes that only unbound cortisol, which is around 5% of total cortisol in non-stressed situation (Lewis et al., 2005), can enter the tissues and the capillary boundaries. In non-stress situations unbound cortisol constitute only 5% of total cortisol, but in regards to the free-hormone hypothesis only this free fraction can infiltrate tissues and circulate through cell membranes into the cytosol to bind the intracellular glucocorticoid receptor with high affinity (Perogamvros et al., 2012).

Circulated cortisol binds to plasma proteins, consisting of albumin (10-15%) and Corticosteroid Binding Globulin (CBG – transcortin - a glycoprotein synthesized in the liver) (80%). Cortisol binds to CBG with high affinity and low capacity whereas to albumin it binds with low affinity and high capacity. The role of cortisol depends on induction of intracellular pathways. In consequence, clarifying which fraction is responsible for cortisol's entry into cells is important in order to identify and study its biological functions, and to relate these functions to circulating and tissue-available concentrations (Perogamvros et al., 2012). CBG is the most important transporter for cortisol and it regulates cortisol bioavailability, accompanied by other mechanisms that modulate systemic and tissue levels of glucocorticoids, such as corticosteroid 11 β -dehydrogenase (Perogamvros et al., 2012; Moisan et al., 2013). CBG plays important roles such as buffer, carrier and delivery molecule. The delivery role of CBG is a biological role that extends beyond that expected of a simple carrier molecule. In particular, CBG is a member of the serine proteinase inhibitors. It goes through conformational change on interaction with target proteinases, which affects its function as a

steroid-binding protein. Cleavage of human CBG by neutrophil elastase produces a conformational rearrangement and substantial loss of cortisol-binding activity. Cortisol has an important role in homeostasis and regulation of energy, which has been investigated in past studies (Chrousos, 1998). Several metabolic processes are mediated by cortisol, including increasing cardiovascular output and respiration, glucose metabolism and cerebral perfusion rate (Lupien et al., 1999). In terms of cortisol receptors, there are two distinct intracellular corticosteroid receptors through which cortisol exerts its effects: glucocorticoid (GR) and mineralocorticoid (MR) receptors (Jurueña et al., 2009). The MR has a high affinity for endogenous glucocorticoids while the GR has a low affinity. Under a stressful situation when the cortisol concentration is high, negative feedback is mediated mainly by GRs in the hippocampus, hypothalamus and pituitary whereas under basal cortisol level MRs in the hippocampus come into play as the main receptors regulating cortisol levels (Jurueña et al., 2009). There is some evidence that blocking GRs may be useful in the treatment of depression (Belanoff et al., 2001; Flores et al., 2006).

1.3.3 Brain

Several parts of the brain have been shown to be involved in MDD (Herbert et al., 2013) consisting of the regions of the frontal lobe such as prefrontal cortex, Brodman's area 25 and anterior cingulate, the amygdala and the hippocampus (Almeida et al., 2003; Pezawas et al., 2005; Sartorius et al., 2007; Kennedy et al., 2011).

Several studies have questioned whether or not cortisol can be considered a crucial factor in part of this brain network (Herbert et al, 2013). Moreover, high concentrations of GRs and MRs are found in the hippocampus which makes it

crucial to investigate the relation between altered cortisol levels and hippocampus structure and volume in MDD (Herbert et al., 2012).

O'Brien et al. (2004) have reported that there is no relation between the hippocampus volume reduction and increased salivary cortisol in MDD patients. In contrast, a study by Dedovic et al. (2010) has shown an association between abnormal regulation of basal cortisol levels and the reduction of hippocampus volume. Specifically, three groups, consisting of control, subclinical and high risk subclinical groups on the basis of Beck Depression Inventory (BDI) scores, underwent a magnetic resonance imaging (MRI) scan (1.5-T) and gave saliva for the evaluation of cortisol (at the time of awakening, 30 minutes after awakening, 60 minutes after awakening, 16:00 p.m., and 21:00 p.m.) during three non-consecutive 24 hour periods when the subjects were working. Results showed that the high-risk subclinical group had a higher CAR and a decreased hippocampus volume compared with control subjects.

1.3.4 Salivary Cortisol

Numerous studies have focused on the evaluation of salivary cortisol in patients with mental illness (Obayashi et al., 2013). Saliva is produced by the parotid gland, the submandibularis gland, and the sublingualis gland and represents an early and reliably collected specimen for the assessment of cortisol (Herr et al., 2007; Obayashi et al., 2013).

Two biological systems play major role in response to a psychological stress: the sympathetic adreno-medullary system and the HPA axis. The secretion of cortisol in saliva is controlled by both systems, though mainly by the HPA axis (Obayashi et al., 2013). Cortisol is detectable in saliva, plasma and other fluids. Although previous studies pointed out that cortisol levels in saliva can be affected by many factors such as gender, age, circadian rhythm, meals, drugs, autonomic function

and salivary gland function (Schwartz et al., 1993; Toda et al., 2004; Nater et al., 2006), with appropriate methodological care cortisol can be evaluated reliably in saliva (Obayashi et al., 2013). Thus, salivary cortisol has become a useful quantitative biomarker for patients with mental illnesses (Toda et al., 2013).

The use of saliva cortisol as a biomarker has a number of advantages. First, no effect of salivary flow rate has been reported on salivary cortisol levels. Second, acute short-term psychological stress does not have an effect on salivary flow rate (Obayashi et al., 2013). Third, physical stress does not produce an important effect on the salivary cortisol level (Vanaelst et al., 2012).

One of the other advantages is that cortisol can be stored for a long term, even at room temperature, and thus can be easily collected at home by subjects and it can represent a more naturalistic measure less affected by experimental procedures in comparison to other potential markers (Naumova et al., 2012). Salivary cortisol collection has been found to be a non-invasive, stress free and less anxiety-provoking method as opposed to blood testing (Desai et al., 2014; Wormwood et al., 2015).

However, there are still methodological issues around measuring cortisol in saliva as there are no clear norms for the level of free cortisol in saliva either at awakening time (range of 4.7–18.5 nmol/l) or 30 minutes after awakening (range of 8.6–21.9 nmol/l) (Clow et al., 2004).

1.3.5 Cortisol Awakening Response (CAR)

1.3.5.1 Overview

The CAR is a rapid increase in the cortisol level within the first hour after awakening (Pruessner et al., 1977; Clow et al., 2004). The CAR is a genuine response to awakening and is distinct from the 24 hours diurnal cortisol profile (Schmidt –Reinwald et al., 1999; Wilhelm et al., 2007; Fries et al., 2009). During

the CAR phase, cortisol concentration increases to a variable extent compared to the cortisol level at the awakening time, reaching its highest point between 30-45 minutes after awakening (Clow et al., 2004; Fries et al., 2009).

The standard clock times such as 9:00 a.m., which were previously used for calculating morning cortisol levels, have been to a large extent replaced by assessment of the CAR, due to the measurement bias in MDD patients whose awakening time often differs markedly from controls.

Previous research conducted in adolescents aged 16 to 18 by Adam et al. (2010) found that the CAR is an important prospective predictor of MDD. In detail, salivary cortisol was collected six times during a 24 hour period over three consecutive weekdays. The time of saliva collections was at awakening, 40 minutes after awakening and around 3, 8 and 12 hours after awakening. The outcome of the study showed that the increased CAR might be a predictive factor for both onset and recurrence of MDD.

However, further research is required to know more about the CAR and its function.

1.3.5.2 The reliability of the CAR

The CAR is recognized as a reliable index of HPA axis activity (Schmidt-Reinwald et al., 1999). In particular, assessing the CAR is a non-invasive test increasingly noted to be a useful way of capturing information about stress reactivity in numerous studies (Pariante et al., 2008; Adam et al., 2010).

It has been argued that the reliability of calculating the CAR is increased when CAR assessments are done consecutively over 48 hours (Wust et al., 2000; Kunz-Ebrecht et al., 2004).

In previous studies, the CAR has been widely investigated either in healthy subjects, or in association with cardiovascular, autoimmune, atopic, allergic, and psychiatric diseases (Wust et al., 2000; Clow et al., 2004).

1.3.5.3 Function of the CAR

The function of the CAR has not yet been fully clarified. It seems that it is associated with anticipation of the demand of the day ahead, supported by evidence showing that the CAR is higher on weekdays compared to weekends. Thus, numerous studies have observed a higher CAR during weekdays (Kunz-Ebrecht et al., 2004; Schlotz et al., 2004; Thorn et al., 2006). The CAR may show changes in association with stress levels and with feeling of happiness (Kunz-Ebrecht et al., 2004). It may also play a role as an adaptive response (Adam et al., 2006).

1.3.5.4 Neural regulation of the CAR

Both the limbic system and the prefrontal cortex are involved in the regulation of the CAR. The other part of the brain that can influence the CAR is the suprachiasmatic nucleus (SCN) (Edwards et al., 2001; Clow et al., 2004; Hucklebridge et al., 2005; Fries et al., 2009; Thorn et al., 2011).

1.3.5.5 Factors affecting the CAR

Previous studies have shown that several factors may influence the CAR, although there have been inconsistent outcomes in relation to these factors. Of note, a significant genetic impact contributing to each subjects' CAR has been shown in previous studies (Wust et al., 2000; Hellhammer et al., 2007).

1.3.5.5.1 Age and Gender

Age and gender are important factors that should be considered. Some studies have found an impact of age on the CAR such as an association between older age and lower CAR (Kudielka et al., 2003), although this may be a statistical error

related to the sample size given that in those studies having a large sample size, no effect of age has been reported (Pruessner et al., 2007).

In regards to gender, a number of studies have found that women have a larger CAR compared to men (Wust et al., 2000; Pruessner et al., 2007). Of note, Kudielka et al. (2003) found that there is a tendency for a delayed cortisol peaking in females as well.

1.3.5.5.2 BMI

An association between Body Mass Index (BMI) and elevated cortisol level has been reported (Bjorntorp et al., 2000). Furthermore, Therrien et al. (2007) assessed the CAR in obese and reduced obese-the obese people who lost weight- men and women; Fifty-one men (16 lean, 19 abdominally obese, and 16 reduced obese) and 31 women (12 lean, 10 subcutaneously obese, and 9 reduced obese) were participated – the groups were defined by strict ranges of BMI and waist circumference; lean (BMI <27 kg/m², waist circumference <100 cm for men and <90 cm for women); obese (BMI 30 to 35 kg/m², waist circumference >100 cm for men and <100 cm for women; and reduced obese (BMI >30 kg/m², waist circumference >100 cm for men and <100 cm for women) , before weight loss, minimal weight loss of 5 kg, still losing weight or just stabilized. Weight loss resulted from changes in eating and physical activity. The results showed that men with visceral obesity showed an increased cortisol response. In particular men with visceral obesity showed an increased cortisol, whereas this response tends to return to normal in a reduced obese state. Reduced obese women presented a significantly increased CAR compared with lean subjects, but there was no difference in this variable between lean and obese subjects. No gender difference was observed in CAR between lean men and lean women.

1.3.5.5.3 Stress

Stress is one of the most important factors that can affect the CAR. For example, there is an association between chronic overload and increased CAR (Schlotz et al., 2004). In particular, the observed weekend-weekday changes in the CAR show that the day of the cortisol assessment is important. Of note, a higher level of perceived stress and worrying is associated with an increased CAR (Wust et al., 2000).

1.3.5.5.4 Smoking

Mixed results have been found about the effects of smoking on the CAR. One study has shown that smoking status is related to the elevated CAR, but found no differences between ex-smokers and never-smokers, suggesting that smoking has a short-term effect on the neuroendocrine system (Badrick et al., 2007).

1.3.5.5.5 Alcohol

Junghanns et al. (2007) pointed out that there is an association between alcohol consumption and CAR. In particular, heavy drinkers (above 28 units of alcohol consumption per week in men and 21 units per week in women) had a higher CAR compared to moderate drinkers (Junghanns et al., 2007).

1.3.5.5.6 Pain

Sudhaus et al. (2007) found an association between the CAR and behavioural pain coping-strategies. In detail, 31 low back patients (chronic and acute) and 14 healthy controls participated in the study. Although there were significant interrelations between awakening responses and the behavioural pain coping-strategies, the groups did not differ in their CAR.

In a subsequent study, Generaal et al. (2014) found a lower CAR in subjects who suffered from multi-site musculoskeletal pain compared to controls. Another

study found that an increased CAR was associated with higher pain intensity and unpleasantness (Goodin et al., 2012).

1.3.5.5.7 Sleep related factors

1.3.5.5.7.1 Time of awakening

Conflicting results have been found about the association of the CAR and time of awakening. Elevated CAR has been reported in subjects who wake up early compared to those waking later (Edwards et al., 2001; Kudielka et al., 2003), although the mode of awakening or waking up at unexpected times did not have an impact on the CAR (Wust et al., 2000).

1.3.5.5.7.2 Sleep duration and insomnia

Wust et al. (2000) presented evidence that in subjects who have shorter sleep duration there is a comparable CAR to those subjects having longer sleep duration.

The findings of research investigating the influence of insomnia on the CAR are inconclusive. Both a lower awakening response in subjects suffering from insomnia (Backhaus et al., 2004), and no effects on the CAR, have been found (Dettenborn et al., 2007).

1.3.5.5.8 Specific time of collection

Okun et al. (2010) has shown that a delay of more than 15 minutes between the wakening time and collecting the first specimen can significantly affect the CAR. As a result, some studies have classified subjects with a delay of 10 or more minutes after awakening as non-compliant (Kunz-Ebrecht et al., 2004).

1.3.5.5.9 Oral contraceptives and menstrual cycle

Pruessner et al. (1997) observed a small effect of oral contraceptive use on the CAR; the study showed that in women who took oral contraceptives, there was a tendency for lower early morning free cortisol levels. However, overall studies

investigating the effect of menstrual cycle on the CAR are inconclusive. Thus, while some studies have found no difference between menstrual phases (Kudielka et al., 2003), Kirschbaum et al. (1999) found that salivary cortisol responses were considerably different between women in the luteal or follicular phase, and that the CAR increases during the luteal phase.

1.3.5.5.10 Burnout and medical comorbidity

An increased CAR has been reported in patients with burnout compared to healthy controls (De Vente et al., 2003), whereas evidence for a lower CAR in both clinical and non-clinical burnout has been found in a study by Oosterholt et al. (2015).

Several other factors can have an impact on the CAR. Upper respiratory illness can reduce the level of the CAR (Edwards et al., 2003), whereas hypertension (Wirtz et al., 2007), atherosclerosis (Hurwitz et al., 2001) and functional gastrointestinal disorders (Bohmelt et al., 2005) can all increase the CAR.

1.3.5.6 Calculation of the CAR

For reporting changes in endocrinological variables, the use of repeated measurements over the time has been introduced into the study protocols in endocrinological research. For detecting a possible association between repeated measures and other variables, the area under the curve (AUC) is regularly used to integrate multiple time points.

The formulas for computation of the AUC have varied between laboratories (Pruessner et al., 2002). Studies have calculated the AUC in two ways, both of which are based on the trapezoid formula, as follows:

1) AUC_g (Area under the curve with respect to the ground): The total cortisol released in the period after awaking. If the initial awaking cortisol concentrations

are high, then the AUC_G will be large even if the increase following awaking is minimal (Edwards et al., 2001).

2) AUC_i (Area under the curve with respect to the increase): This is the CAR measured as the increase from baseline and it is not related to the actual cortisol levels at awakening. This measure of the CAR is said to be related to the sensitivity of the system (Chida et al., 2009).

1.4 Biomarkers in Depression

1.4.1 Overview

Depression is known as a debilitating disorder, which currently lacks well-known biomarkers of aetiology and treatment response. To understand the complexity of depression and to develop new therapeutic targets, it is important to examine several aspects of the potential biological components of depression. Chief among the potential biomarkers are (1) genetic factors; (2) brain changes found with neuroimaging; and (3) endocrinological dysfunction, in particular alterations to the HPA axis.

Numerous previous studies have been carried out to explore these factors in MDD patients. Many of these studies were undertaken in order to find novel biomarkers for MDD, and several studies have also attempted to look at the interaction of these factors.

1.4.2 Genetics

1.4.2.1 BDNF (Brain-Derived Neurotrophic Factor) and BDNF Val66Met polymorphism

BDNF, a protein that is a member of the neurotrophic family of growth factors, is the most abundant neurotrophin in the brain (Hong et al., 2011). It is an important factor controlling the growth of new neurons and synapses, and the proliferation and survival of neuronal cells (Acheson et al., 1996). Thus, the regulation of

synaptic plasticity and connectivity is associated with BDNF (Verhagen et al., 2010). It is active in the brain in the areas relating to memory, learning, and higher thinking. Therefore, there is a high concentration of BDNF in the hippocampus and cerebral cortex (Yamada et al., 2003).

Previous studies have investigated the val66met polymorphism, a single nucleotide polymorphism in the BDNF gene that is due to variation between valine and methionine (Montag et al., 2009). This polymorphism has an effect on cellular processing, trafficking and activity dependent-secretion of BDNF (Hong et al., 2011). It seems a suitable factor for investigation to find a link between the BDNF gene and MDD (Hong et al., 2011).

BDNF is associated with memory function in the brain. Poor memory performance, which is a symptom of depression (Veiel et al., 1997), is related to the met allele (Egan et al., 2003). Associated with this, the crucial role of BDNF in learning and memory has been shown in some studies (Bekinschtein et al., 2008; Lu et al., 2008). Due to its important effects on synaptic plasticity, it seems that BDNF is necessary for short term and long-term memory storage (Alonso et al., 2002; Bekinschtein et al., 2008).

A decrease in BDNF expression may play a major role in the pathophysiology of MDD (Karege et al., 2002; Duman et al., 2004; Schumacher et al., 2005; Angelucci et al., 2005; Lang et al., 2005). Peripheral BDNF levels are reduced in MDD patients (Sen et al., 2008). Karege et al. (2002) found that the level of BDNF in the serum of MDD patients is considerably lower than healthy controls. Animal and clinical studies have demonstrated the important role of BDNF in MDD and antidepressant drug action (Hong et al., 2011). An antidepressant-like influence in an animal model of depression has been observed by infusion of BDNF into the midbrain (Siuciak et al., 1997). Associated with this, it has been suggested

that BDNF up regulation in the hippocampus of MDD patients may be a result of using antidepressants (Shimizu et al., 2003; Duman et al., 2004; Angelucci et al., 2005; Lang et al., 2005;) and that increasing BDNF levels may play an important role in antidepressant efficacy (Santarelli et al., 2003). Antidepressants such as fluoxetine have been observed to increase BDNF levels in the hippocampus (Herbert et al., 2012).

Associated with these results, the BDNF val66met polymorphism seems to have a significant effect on the function of neural plasticity in the medial temporal lobe (Montag et al., 2009).

The BDNF val66met polymorphism has a major role in the generation of spontaneous strategies during navigation behaviour in healthy controls. Several memory systems are involved in parallel processing of spatial information during navigation. A number of studies have determined between hippocampus-dependent 'spatial' navigation, which relies on knowledge of the relationship between landmarks in one's environment to build a cognitive map, and habit-based 'response' learning, which needs the memorization of a number of actions and is regulated by the caudate nucleus. Studies have demonstrated that people spontaneously use one of these two alternative navigational strategies with approximately equal frequency to solve a given navigation task, and that strategy correlates with functional magnetic resonance imaging (fMRI) activity and grey matter density (Banner et al., 2011). In association with this, increased hippocampal activation in the val group compared with the met group during a virtual navigation task has been found in a study. They showed that met carriers in compared to val homozygote used response strategy rather than spatial navigation (Banner et al., 2011). This has led to a suggested relationship between the BDNF val66met polymorphism and depressed patients, who consistently

show impaired spatial navigation (Cornwell et al., 2010). The evidence that the BDNF val66met polymorphism cannot fully explain differences in spontaneous learning strategies by itself supports the idea that some other actors, such as stress or polymorphisms in other genes, may have additional roles mediating between genotype and behaviour (Banner et al., 2011). The influence of BDNF on learning and memory strategies may be affected by exposure to stress as well (Song et al., 2006; Heldt et al., 2007).

Bus et al. (2012) investigated the effect of gender on serum BDNF levels in healthy controls. In males, higher serum levels of BDNF were found in met carriers than val/val homozygotes whereas in females no effect of val66met genotype was found. In line with this, Verhagen et al. (2010) suggested that in MDD, the BDNF val66met polymorphism may play a more important role in men than women. These results (Verhagen et al., 2010; Bus et al., 2012) may suggest that the inconsistent findings of earlier results may have been influenced by male-female ratios in previous studies.

In sum, it has been argued that a lack of statistical power, small sample sizes and failure to account adequately for the influence of gender have contributed to the present controversies about the role of BDNF and the val66met polymorphism in MDD (Verhagen et al., 2010).

1.4.3 Structural changes of the brain

Volumetric changes have been found in a number of brain areas in MDD patients compared to healthy controls in previous studies, with suggestions that structural brain alterations may play an important role in MDD (Arnone et al., 2012; Samann et al., 2013).

Structural MRI is a key methodology that has been widely utilised to identify key regions of the brain having an important role in the pathophysiology of MDD (Drevets et al., 2001; Fitzgerald et al., 2008; Rigucci et al., 2010).

Reduction of the grey matter volume in MDD has been observed in numerous studies, and specifically in regions within the limbic-cortisol circuits (Sacher et al., 2011). Significant structural brain changes may be especially apparent in patients with severe or chronic depressive disorders. For example, Serra-Blasco et al. (2013) studied 66 MDD patients and 32 healthy controls. All patients were taking several medications at the beginning of the study. The patients consisted of 3 groups: a chronic depressive/ treatment resistant group; a euthymic group with HAM-D 17 scores < 8 for the past 6 months but with three or more previous episodes of MDD; and patients with a first episode of depression. The study suggested that there is a negative effect of long-lasting depression on the grey matter, and in particular that there is an association between smaller frontotemporolimbic volumes and the duration of MDD.

1.4.3.1 Amygdala

Although, it has been shown that the amygdala has an important role in the pathophysiology of MDD because of its significant action in the process of memory and emotion in the brain (Hajek et al., 2009), the role of amygdala volumetric changes in MDD is still unclear.

Results from studies investigated the structure of the amygdala in MDD have been inconsistent (Sheline et al., 2000; Hamilton et al., 2008; Lorenzetti et al., 2009). Cohort studies with MDD patients have suggested either increased (Frodl et al., 2002; Frodl et al., 2003; Lange et al., 2004; Weniger et al., 2006; van Eijndhoven et al., 2009), reduced (Hastings et al., 2004; Tang et al., 2007; Frodl

et al., 2008); or unchanged amygdala volumes (Morys et al., 2003; Caetano et al., 2006; Monkul et al., 2007).

Associated with this, conflicting results have been observed in studies that have investigated a variety of potential moderators of the association between amygdala volume and MDD, including laterality, gender, medication and clinical factors such as severity and duration, number of episodes, age of onset and the presence of psychiatric illness in the family (Sheline, 2000; Bass et al., 2004; Hamilton et al., 2008; Lorenzetti et al., 2009). In detail, considering the influence of medication on amygdala volume in MDD patients, Hamilton et al. (2008) argued that studies which used medicated subjects indicated that amygdala volume was significantly increased in depressed compared to healthy controls, whereas studies using unmedicated depressed subjects showed a reliable decrease in amygdala volume in depression. Regarding clinical factors, amygdala grey matter volume reduction has been found in first episode of depressed patients, who were mostly drug free, in compared with chronic patients and controls (Bora et al., 2011). Further, it has been argued that amygdala size may vary in association with illness duration, while age at onset does not seem to have a major role. In detail, while unipolar patients earlier in the course of illness tend to have increased amygdala volume, depressed patients with a longer illness duration and with higher number of MDD episodes tend to show volumetric reductions (Lorenzetti et al., 2009). In regards to laterality, it is not clear whether MDD affects the left and right amygdala in the same way (Lorenzetti et al., 2009); In details, Baas et al. (2004) by combining results among 54 fMRI and Positron Emission Tomography (PET) studies in a meta-analysis showed that the left and right amygdala may have important but not similar roles in the pathophysiology of MDD. The left amygdala is more often activated than the right

amygdala; which means they observed a strong preponderance of left amygdala activations over right amygdala activations in functional neuroimaging studies of emotion processing. In terms of the laterality of amygdala volume changes, Lorenzetti et al. (2009) observed a decreased left amygdala volume in remitted patients compared to healthy controls. In contrast, Bremner et al. (2000) found a decreased right, but not left, amygdala in remitted depressed patient compared to controls. Associated with this, Caetano et al. (2004) found a trend towards smaller left amygdala volumes in MDD patients compared to healthy controls. Importantly, it is not clear whether volumetric alterations of the amygdala are a state or trait related to MDD (Lorenzetti et al., 2009). Thus, Eijndhoven et al. (2009) have shown that larger amygdala volumes could be a state marker of MDD, specifically in the early onset of MDD. Supporting this, another study conducted by Lorenzetti et al. (2010) showed that the alteration of amygdala volume is state related as remitted patients had a larger amygdala volume compared to currently depressed patients. In particular, they observed larger amygdala volume in remitted patients who were antidepressant free for six months. This study did not show a significant difference between current depressed patients and healthy controls. It has been argued that increased left amygdala volume in remitted MDD patients may be a neurobiological marker of vulnerability to relapse, or may represent recovery from MDD. In this study, in terms of using antidepressants, they found that patients who were not taking medication during the past 6 months either they were the current MDD or the remitted MDD subjects, showed larger left amygdala volumes when compared to healthy controls. Although they did not observe an interaction between diagnosis and medication, significantly less remitted MDD participants were taking medication when compared to the current MDD subjects. Therefore, medication

may cause to an increased left amygdala size in the remitted MDD group compared to the healthy controls and to a non-significant trend for a larger amygdala compared to the current MDD patients.

In contrast with this, another study found both amygdala to be considerably enlarged in currently depressed patients, but no difference between remitted patients and control subjects (Eijndhoven et al., 2009). Associated with this, in another study of patients with melancholic depression, patients with psychotic depression and normal controls, although they did not find any change in hippocampus volume, or anterior and posterior subgenual cortex, between 3 groups; larger amygdala volumes of both patient groups were observed compared to controls (Vasilopoulou et al., 2011)

1.4.3.2 Hippocampus

Hippocampus volume reduction is one of the most frequently observed structural brain changes in MDD patients (Campbell et al., 2004; Mak et al., 2009; Koolschijn et al., 2009). Cole et al. (2010) reported deformations in the subiculum and the CA1 subfield extending into the CA2-3 subfields in the tail regions of both hippocampi in MDD patients compared to healthy controls. More recently, meta-analyses have shown reduced hippocampal volume in first episode depression (Cole et al., 2011) and increased hippocampal volume in remitted depressed patients compared to currently depressed patients (Kempton et al., 2011).

Hippocampal changes may be of clinical relevance. Thus, Frodl et al. (2008) has shown that lower hippocampus volume forecast a worse clinical outcome three years after hospitalization in remitted patients in compared to non-remitted patients.

1.4.3.3 Prefrontal cortex and other brain areas

The reduction of grey matter volume in the fronto-limbic and other brain areas is one of the most important findings in MDD (Sacher et al., 2011, Serra-Blasco., 2013). Numerous studies have reported grey matter reduction in orbitofrontal cortex (Scheuerecker et al., 2010), anterior cingulate gyrus (Tang et al., 2007) and temporal gyrus (Mak et al., 2009; Scheuerecker et al., 2010). In particular, they reported alterations in the superior temporal regions such as decreased volume in the superior temporal gyri bilaterally and in the left planum temporal in both current MDD patients and remitted MDD patients when compared with healthy controls (Takahashi et al., 2010b) and a decreased volume in the left insula of both current MDD and remitted MDD when compared with healthy controls (Takahashi et al., 2010a). Previous studies found alterations in the thalamus (Kim et al., 2008; Vasic et al., 2008), in particular shorter interthalamic adhesion, in current MDD when compared with healthy controls (Takahashi et al., 2009).

Subsequent meta-analyses have also found grey matter volume changes in a number of brain regions such as frontal limbic and thalamus (Du et al., 2012; Bora et al., 2012). In association with these changes, Koolschijn et al. (2009) found a volume reduction in frontal lobes, orbitofrontal cortex, prefrontal cortex, and putamen in MDD patients compared with healthy controls. In line with this, volume reduction of caudate globus pallidus, thalamus and gyrus rectus were found by Kempton et al. (2011). Sacher et al. (2011) showed a lower grey matter volume in dorsolateral frontal cortex and the right paracingulate cortex.

In contrast with these findings, previous studies have shown increases during time in the volume of grey matter within the caudate nucleus and hippocampus, and these increases were related to increased positive mood and increased

learning (Starkman et al., 2003; 2007). In line with this, Starkman et al. (2007) argued that decrease of cortisol concentration in Cushing's syndrome patients is significantly related with decrease in the degree of depression and the depressive syndrome, although there was no significant relationship between change in the depression subscale and change in urinary free cortisol concentration in the study (Starkman et al., 2007). This suggests that the effects of cortisol are less a direct linear effect on mood and more the result of the interaction of cortisol with caudate structure and function (Starkman et al., 2003; 2007).

Taken together, previous studies found morphometric changes that could be argued to be either state- related brain changes, such as shorter interthalamic adhesion, or vulnerability trait markers, such as decreased insular volume in the medial prefrontal network.

Interestingly, focusing on a specific region, one of the regions which its volume alteration has been most frequently observed in MDD patients (Klauser et al., 2015) is the anterior cingulate cortex (ACC). The ACC is a part of the medial prefrontal network that has been shown to have crucial role in emotional processing. This medial prefrontal network surrounds the medial part of the orbito-frontal cortex – also known as ventromedial prefrontal cortex (vmPFC) – and can be broadened to limbic regions as well as to basal ganglia and thalamus to form an extended medial prefrontal network. In regards to ACC volume changes, previous studies have found reduced ACC volume in MDD patients compared to healthy controls (Tang et al., 2007; Frodl et al., 2008; Koolschijn et al., 2009; Abe et al., 2010). Furthermore, considering the effect of clinical factors, an association between a longer duration of illness and grey matter volume reduction in the rostral ACC has been found (Fornito et al., 2009; Ellison-Wright et al., 2010; Bora et al., 2011). In a recent study of grey and white matter in

patients with a life time history of MDD, ventromedial prefrontal cortex (vmPFC) grey matter bilateral reduction in remitted MDD patients when compared with currently depressed and healthy controls was found. These findings, therefore, support the argument that cortico-limbic disturbances may contribute to the underlying pathophysiology of MDD (Klauser et al., 2015).

Interestingly, in regards to impacts of antidepressants, grey matter changes have been investigated as predictor of antidepressant treatment response in MDD patients in a number of previous studies (Samann et al., 2013). In regards to importance of early treatment response, biomarkers are essential to help to find patients that are less likely to respond to standard antidepressant treatment and other therapies at an early stage of the treatment. Samann et al (2013) found that the cortex volume of certain brain areas such as left hippocampus, right lateral temporal cortex, and subcallosal/orbitofrontal cortex is significantly associated with different responses to treatment through gender-by-region interactions. A hippocampal /temporolateral composite marker showed robust changes in both first episode and recurrent unipolar patients.

Smith et al. (2012) showed that there is an association between volume increases of the left dorso lateral prefrontal cortex (DLPFC) with SSRI-treated depressed subjects over a 12 week period. However, In terms of the impact of antidepressant use, it is presently unclear, whether antidepressant treatments might have significant effects on regional grey matter volumes and whether these potential structural influences could be associated with their efficacy in treating clinical symptoms.

In regards to BDNF , previous studies have shown, stress reduces the expression of BDNF, and other trophic factors, in brain areas associated with emotion and mood; and this could play a role in the cortical atrophy and vulnerability to relapse

seen in mood disorders. It has been shown that antidepressant therapy alters or blocks these influences (Duman et al., 2006). Increases in BDNF due to antidepressant therapy can cause dendritic arborization and increased numbers of synaptic contacts and spines, and rises in the development and plasticity of neural networks (Castren et al., 2010). It is possible that such synaptogenesis and increased dendritic arborization could cause increases in local grey matter volume.

1.4.4 Genetics and Neuroimaging

Heritability estimates for MDD are of the order of 48-75% (McGuffin et al., 1996). The combined use of neuroimaging tools and genetic methods to study mood disorders has increased over recent years. It is hoped that combining these two areas will advance our insight into the brain and refine our understanding of MDD, subsequently causing improved treatments. In association with this direction, investigating the effects of the val66met BDNF polymorphism, a valine (val) to methionine (met) substitution in the 5' pro-region of the human BDNF protein – as a genetic factor – on structural amygdala changes – as a neuroimaging factor – in MDD patients could give a new insight in understanding of MDD.

1.4.4.1 BDNF val66met polymorphism and structural changes in the brain

It has been found that the val66met polymorphism may have a significant influence on the volume of several parts of the brain, such as amygdala, hippocampus and parahippocampal gyrus (Pezawas et al., 2004; Bueller et al., 2006; Montag et al., 2009).

Interestingly, Montag et al. (2009) has shown an association between the val66met allele, parahippocampus, and right amygdala, in which met allele carriers had a smaller amygdala and parahippocampus.

Numerous studies have focused on hippocampal volumetric changes (Egan et al., 2003; Harrier et al., 2003; Pezawas et al., 2004; Kanellopoulos et al., 2011). Although, many studies have shown no association between hippocampus volume alteration and the val66met gene polymorphism (Jessen et al., 2009; Karnik et al., 2010; Benjamin et al., 2010; Cole et al., 2011), for example, Harrisberger et al. (2015) in a meta-analysis suggested that there is no association between this BDNF polymorphism and hippocampal volumes. For each BDNF genotype, the hippocampal volumes were significantly lower in neuropsychiatric patients than in healthy controls.

Hajek et al. (2012) in a meta-analysis observed an increased hippocampal volume in val homozygotes compared to the met-allele carriers in healthy controls (Hajek et al., 2012). This finding supports an effect of the BDNF val66met polymorphism on hippocampal volume changes, presumably due to decreased BDNF secretion. Considering these studies in more details, Egan et al. (2003) examined the influence of val 66met polymorphism on hippocampus and memory function. The met allele was associated with poorer episodic memory, abnormal hippocampal activation assayed with fMRI, and lower hippocampal n-acetyl aspartate (NAA), assayed with MRI spectroscopy. Neurons transfected with green fluorescent protein (met-BDNF-GFP) showed lower depolarization-induced secretion, while constitutive secretion was unchanged. In line with this finding, met-BDNF-GFP did not localize to secretory granules or synapses. These results showed BDNF and its val/met polymorphism could affect memory and hippocampal function; furthermore, it has been argued val/met exerts these effects by influencing intracellular trafficking and activity-dependent secretion of BDNF (Egan et al., 2003; Pezawas et al. 2004; Hariri et al., 2003; Kanellopoulos et al., 2011).

Consistent with the results of studies confirming the effects of val66met polymorphism on hippocampus volume, healthy met carriers with high trait depression showed a decreased hippocampal grey matter volume compared to subjects that were homozygotes for the val allele (Joffe et al., 2009). One study in both healthy controls and MDD patients has shown that carriers of at least one met allele have significantly smaller hippocampus volume (Frodl et al., 2007).

Few studies have been undertaken in relation to right compared to left hippocampus volume alteration in association with the val66met BDNF polymorphism. However, val/val MDD patients showed decreased left hippocampal volume compared to right in a study by Gonul et al. (2011).

When considering other regions of the brain, a smaller volume of the thalamus, fusiform gyrus and several parts of the frontal gyrus were observed in met allele carriers (Montag et al., 2009).

Interestingly, one study in young Chinese adults by combining cortical thickness analysis and voxel based morphometry (VBM) analysis found grey matter reduction in frontal, temporal, cingulate and insular cortex in the met/met group in comparison with a val/val group. In fact, by combining cortical thickness analysis and VBM, the specific role of the met/met genotype could be assessed in detail for the first time, because of a higher frequency of the met/met genotype (27.9%) (Yang et al., 2012).

1.4.5 Early life stress and major depressive disorder

The link between stressful life events and MDD has been well documented (Cleare et al., 1995). Nemeroff et al. (1999) has argued that the biological basis of the association between early life stress and MDD could be related to constant activation and hyper responsiveness of the hypothalamic and extra hypothalamic corticotropin-releasing circuits and alterations in other neurotransmitter systems

involved in the regulation of the stress responses (Nemeroff et.al, 1999; Heim et al., 2001). In line with this, it has been suggested that neuroanatomical changes of the brain such as hippocampal volume alterations could play a role in the association between early life stress and MDD (Heim et al., 2008).

Epidemiological studies have demonstrated considerable evidence to show a link between early life stress and a significantly increased risk of developing depression (Heim et al., 2008). In line with this, a strong dose-response relationship between the number of childhood abuse experiences and general mental health in adulthood has been shown before (Edwards et al., 2003).

Of note, not all forms of depression are related to early life stress. Some evidence suggests the existence of biologically identifiable subtypes of depression as a function of childhood trauma (Heim et al., 2008). Furthermore, early-life stress is certainly not the only factor causing HPA axis dysregulation in depression, but probably interacts with other risk factors, such as genotype variations and gender. For instance, the dominance of women in depression might be due to gender differences in the prevalence of childhood abuse. In the same way, Heim et al. (2010) has suggested that the effect of gender can be an important factor in the response to childhood abuse. It has been argued that women are more vulnerable to the impact of stressful life events. In line with this, overall rates of MDD in women who have suffered from early life stress are 4 times higher than in women without a history of childhood abuse (Heim et al., 2000).

It has been suggested that the potency of acute life stress as a risk factor for depression depends on the presence or absence of early life stress. Also, symptom patterns and the clinical course of depression may differ as a function of childhood abuse. In particular, childhood trauma has been consistently related to early onset of depression as well as a larger number of episodes or more

chronic depression (Heim et al., 2001; 2008). In association with this, Brown et al. (1994) have shown that there is a relation between the chronicity of depression and both family violence and sexual abuse experienced in early childhood.

In fact, biological and symptom patterns as a result of childhood abuse might extend over DSM diagnostic categories, giving insight into the nature of comorbidity in depression, for instance with anxiety disorders and substance abuse (Heim et al., 2008).

Depressive disorders are recognized to show substantial variability in terms of clinical presentation, onset, course, neurobiological changes, and treatment response, suggesting a heterogeneous group of etiologically distinct disorders. In regards to the past, a binary model of depression prevailed for many years that distinguished between endogenous/psychotic and reactive/neurotic subtypes of depression. In contrast, it is likely that several manifestations of depression are located along a continuum of a single subtype with a psychobiological final common pathway. DSM-III introduced descriptive classification dependent on phenomenology seemingly representing a research-based medical model rather than a clinical-based biopsychosocial model. There is an argument that this current classification has hampered depression research and delayed the discovery of consistent neurobiological findings or predictors of treatment response, and that instead paradigms using all subtypes of depression should be experimentally examined using clinical, etiological, neurobiological and genetic variables. It has been argued that developmental factors should be noted when deriving new depression models. A new typology of depression, based on genetic factors, developmental pathways, and neurobiological patterns might lead to improved diagnosis and treatment, and the identification of reliable predictors of treatment response (Heim et al., 2008).

In line with this, the fact that the impact of childhood trauma may be associated with the subtype of depression, and the evidence that not all individuals who have experienced childhood abuse suffer from MDD, suggest that the association between early life stress and depression is complicated.

Previous neurobiological studies of depression have often not measured or controlled for the effects of childhood abuse. Because of the high prevalence of early life stress among MDD patients, and to a lesser degree in healthy controls, a number of studies did not adequately differentiate the effect of childhood abuse and the effect of MDD. Therefore, the probable influence of early life stress have not been noticed; and an effect of early life stress may have been considered only as an influence of MDD. In line with this, previous results about the neurobiology of depression might be affected by the effect of early life stress, and this hidden influence may explain the conflicting results of the studies. For instance, in studies in which groups only based on presence or absence of MDD, there were no significant influence in regards to stress responsiveness whereas when defining groups based on childhood abuse and MDD, highly significant effects appeared, only in MDD patients with childhood trauma, showing changes in stress response systems (Heim et al., 2004; 2008).

Interestingly, there is an association between childhood abuse and remission rates in MDD. Thus, prospective studies have shown that depressed subjects with a history of childhood abuse are less likely to have a full response to treatment than subjects without a history of childhood abuse (Zlotnick et al., 1997).

Regarding the consequences of MDD, there is a strong association between the risk of attempted suicide and any kind of childhood abuse in chronic and treatment resistant depression (Tunnard et al., 2014), while previous studies

have pointed out that the exposure to early life stress significantly increases the risk of suicide (Dube et al., 2001).

1.4.5.1 Types of early life stress and major depression disorder (Emotional, Physical, Sexual, and Neglect)

The high prevalence of childhood abuse, estimated at around 3 million cases in the U.S. each year, reinforces the importance of conducting research into early life stress. Numerous epidemiological studies have assessed rates of exposure to early life stress to be 25-45% (Costello et al., 1998; McCloskey et al., 2000). More recently, a retrospective self-report study of community adults found that the prevalence of childhood abuse was 30% in women and 41% in men (Scher et al., 2004). In particular, the breakdown of abuse into subtypes has been reported to be about 60% neglect, 20% physical abuse, and 10% sexual abuse (Children's Bureau, Administration of Children, Youth, and Families, 2006).

In line with this, Briere et al. (2003) investigated the prevalence of childhood abuse through a mailed questionnaire to the general population in the U.S. From 1,442 randomly contacted subjects, 935 (64.8%) replied completely. 66 men and 152 women (14.2% and 32.3%, respectively) reported childhood sexual abuse, and 22.2% males and 19.5% females fulfilled the criteria for physical abuse.

Considering sexual abuse, several studies have suggested that around 20–25% of women and 8-9% of men have experienced sexual abuse before the age of 18 (Gorey et al., 1997; McCauley et al., 1997; Holmes et al., 2005). Moreover, 28% of the girls suffered from sexual abuse between the age of 6-16 and 16% experienced physical abuse (Horowitz et al., 1997).

Previous studies showed that the type of abuse and increasing severity, duration and frequency of abuse increases the risk of developing depression (Sedney et al., 1984; Briere et al., 1988; Murphey et al., 1988; Bifulco et al., 1991; Mullen et

al., 1993). In particular, physical abuse is related to post traumatic stress disorder (PTSD) and the severity of anxiety disorder, whereas sexual abuse is more related to panic disorder, agoraphobia, and obsessive-compulsive disorder. In line with this emotional abuse is associated with social phobia combined with substance abuse; and emotional neglect is generally related to the severity of psychopathology (Carr et al., 2013).

Numerous studies have shown the important role of emotional abuse, sexual abuse, physical abuse and neglect in the pathophysiology of depression (Nestler et al., 2002; Heim et al., 2011; Davidson et al., 2012). For example, Heim et al. (2000), in a study of 49 healthy women aged 18 to 45, showed that hyperactivity of the HPA axis and autonomic nervous system is an important consequence of early life stress, and in particular, physical and sexual abuse. Specifically, this study recruited four groups of women with regular menses, and without a history of mania or psychosis. The four groups comprised: 13 subjects with a history of current MDD who also had a history of sexual and physical abuse, 10 subjects with current MDD but without a history of abuse, 14 subjects without MDD but with a history of sexual or physical abuse, and 12 subjects without a history of abuse or any psychiatric disorder. Further exclusion criteria were a history of substance abuse or eating disorders within 6 months, and the use of psychotropic or hormonal medication. Women with a history of childhood abuse and a current MDD showed a more than 6-fold higher ACTH response to stress than age-matched controls.

Another study in almost 2000 women suffering from depression and anxiety showed that levels of psychological problems and physical symptoms and the number of suicide attempts is higher in women with a history of physical and

sexual child abuse compared to women without a history of physical and sexual abuse (McCually et al., 1997).

Returning to clinical factors, it has been reported by Carr et al. (2013) that there is an association between chronicity of depression and childhood physical and sexual abuse. It has been argued that there is a link between the severity of MDD and these two types of abuse as well (Carr et al., 2013). Sexual abuse may also be related to an earlier age of first onset of depression. Carr et al. (2013) argued that there is an association between emotional abuse and social abuse. Specifically, there is a link between emotional neglect and the early onset of depression, depressive symptoms and chronicity of depression.

1.4.6 Early Life Stress, HPA axis and cortisol awakening response

The existence of association between cortisol levels and early life stress has been found in a number of studies (King et al., 2001; Cicchetti et al., 2001; Lupien et al., 2009).

For instance, Lemieux et al. (1995) found increased 24 hours urinary cortisol excretion in females with a history of childhood sexual maltreatment and PTSD. In contrast, Stein et al. (1997) in women with a history of childhood sexual abuse observed hyper suppression of salivary cortisol concentrations in response to a low dose of dexamethasone, although no effect on basal morning cortisol concentration in women was observed. However, decreased concentrations of basal plasma salivary cortisol in the morning have been found in abused women in other studies (Heim et al., 2001).

Associated with this, King et al (2001) investigated the basal functioning of the HPA axis by measuring morning salivary cortisol in relation to early sexual abuse in girls aged 5 to 7 years. The study showed that abused subjects had

significantly lower cortisol in comparison to control subjects, suggesting that children may have an impaired HPA axis after childhood abuse.

However, it is not well documented whether the effect on cortisol levels continues into adulthood, due to few longitudinal studies having been undertaken (Power et al., 2012). Nevertheless, a number of studies have suggested that severe childhood abuse can impact on cortisol secretion in adulthood, where it presents as hypocortisolemia in adulthood (Stein et al., 1997; Gunnar et al., 2001; Roy et al., 2002; Weissbecker et al., 2006; Power et al., 2012). Furthermore, Power et al. (2012) demonstrated that in a non-clinical population, flattened morning cortisol secretion is related to the degree of cumulative early life stress in childhood.

It has been suggested that the specific type of early life stress may have an impact on the patterns of cortisol secretion (Lupien et al., 2009). Thus, low glucocorticoid concentrations seem to develop in early childhood in response to trauma, raising the possibility that these low cortisol levels in turn render an individual vulnerable to developing PTSD in response to trauma in adulthood. However, arguing against such a pathway, another study by Peng et al. (2014) argued that childhood neglect may cause hyperactivity of the HPA axis. This study consisted of 28 MDD patients with childhood neglect and 30 MDD patients without childhood neglect, who were compared with 29 age and gender matched healthy controls without childhood neglect and 22 control subjects with childhood neglect. The change in cortisol levels between the time of awakening and 30 minutes after awakening was used as the measure of the HPA axis reactivity. They found childhood neglect may cause hyperactivity of the HPA axis functioning and also dysfunctional attitudes, but does not affect the severity of depression.

In line with this, other studies of adults who have experienced childhood abuse show hyper-reactivity of the HPA axis in abused, depressed subjects and hypoactivity in subjects with PTSD. The changes in abused, depressed adults have been associated with CRH-induced escape of glucocorticoid secretion from suppression by treatment with dexamethasone, showing that the glucocorticoid feedback of the HPA axis is impaired with increased hypothalamic drive. Therefore, a decreased capacity of glucocorticoids to inhibit the HPA axis when it is stimulated could further accentuate CNS responses to stressors (Lupien et al., 2009).

Considering the CAR as an index of the HPA axis, Engert et al. (2010) argued that there is an association between both increased CAR and increased afternoon cortisol and a history of low parental care. Specifically, 58 subjects consisting of 19 men and 39 women aged 18 to 30, were selected by psychological questionnaires. Saliva cortisol was collected at awakening, 30 min afterwards and at 3 p.m., 6 p.m., and 9 p.m. on three non-consecutive working days. The participants with low parental care showed an increased CAR and afternoon cortisol level compared to those who had received high parental care.

1.4.7 HPA axis, cortisol and cortisol awakening response

One of the most widely replicated and potentially important results of previous studies in MDD patients is the alteration of the HPA axis (Carroll et al., 1976, Young et al., 2001), which is one of the most crucial findings in psychoneuroendocrinology (Arborelius et al., 1999; Nestler et al., 2002). Although numerous studies have shown that there is a relation between hyperactivity of the HPA axis and MDD, and hypercortisolemia in MDD patients has been observed in several previous MDD studies (Gold et al., 1985; Holsboer et al., 1985), for example a study by Vreeburg et al. (2009) showed a higher CAR

among both subjects with current MDD and subjects with remitted MDD, and argued that this may be indicative of an increased biological vulnerability for depression (Vreeburg et al., 2009). One meta-analysis pointed out that there is no strong relation between cortisol and depression (Knorr et al., 2010).

A number of cross-sectional studies have shown an increased cortisol in MDD patients (Young et al., 2006; Vinberg et al., 2008; Dougherty et al., 2009; Vreeburg et al., 2010; Carnegie et al., 2013). In association with this, an increased level of cortisol has been observed in some cross-sectional studies of subjects at high risk, such as having familial history of depression, in MDD (Young et al., 2006; Vinberg et al. 2008; Dougherty et al., 2009; Vreeburg et al., 2010). In particular, higher basal level of cortisol and higher cortisol after both 25 mg and 5 mg dexamethasone intake (Young et al., 2006); higher evening cortisol level (Vinberg et al., 2008); elevated morning cortisol (Dougherty et al., 2009); and higher morning cortisol (Vreeburg et al., 2010). In sum, the cross sectional studies have shown an association between increased CAR and MDD but could not show cause or tease out the aetiology.

The evidence that pathological and iatrogenic hypercortisolaemic states are associated with a high prevalence of depressive symptoms (Patten et al., 2000; Pereira et al., 2010) suggests the hypothesis that the abnormal HPA axis function may be a causative factor in the increased risk of subsequent MDD, although the findings from this large prospective cohort study could not confirm the hypothesis that the CAR, or other basal salivary cortisol measures consisted of cortisol at each time point, diurnal drop and area under the curve, increase the risk of developing a depressive illness (Carnegie et al., 2013).

It has been pointed out that several factors might contribute to the observed HPA axis alterations in MDD patients. Considering age and gender, a number of

studies have suggested that gender may have an important role. In line with this, Deuschle et al. (1997) and Linkowski et al. (1985) have found the higher 24 hours mean cortisol levels in male unipolar and bipolar MDD patients compared to male healthy controls, although no significant difference in 24 hours mean ACTH level was observed. In line with this, Young et al. (2001) has suggested that elevated cortisol levels—ten minute sampling for ACTH and cortisol was performed for 24 hours—are found only in 24% of premenopausal women in compared to healthy premenopausal control women. Recently, the role of age has been investigated. One study argued that older MDD patients show higher rates of abnormal HPA axis activity when compared with younger MDD patients (Murri et al., 2013). In line with this, in a study of older age MDD patients, Bremner et al. (2007) has demonstrated that MDD in late life is associated with both hyper and hypocortisolemia. Specifically, the study consisted of 1185 subjects aged 65 and older. The morning cortisol plasma level was measured. The result of the study suggested that the association between cortisol and MDD is U-shaped rather than linear. The U-shaped relation of cortisol and MDD implies that there is an optimal range of cortisol values associated with the lowest depression rates that is located at the trough of their study's curve. On the other hand, they found clear evidence of a U-shaped relation between plasma cortisol and late-life MDD, but not subthreshold depression. The findings may show that in the elderly, depression is related to an imbalance in stress homeostasis causing either hypoactivity or hyperactivity of the HPA axis. Hypocortisolemic depression was associated with female sex, joint disease, and smoking, and the relation between low cortisol levels and depression was most notable in recurrent depression. Hypercortisolemic depression was related to male sex, older age, cardiovascular disease, current use of NSAIDs, and cognitive impairment. Of note, the majority

of cortisol values they found were within the normal range of 150-700 nmol/L (4% <150 and 12.5% >700). They defined hypocortisolemic and hypercortisolemic depression relative to one another, that is, lowest, middle, and highest tertile.

In agreement with this, Herbert et al. (2012), in a study of the relationship between the BDNF val66met polymorphism and morning cortisol level, also found evidence that the relation between cortisol and MDD may be U-shaped rather than linear. Their study consisted of 279 premenopausal women who were at high risk of MDD. Morning salivary cortisol was calculated for up to 10 days. Subjects were observed for about 12 months by telephone calls at 3-4 monthly intervals. There was a significant U-shaped association between adjusted morning cortisol levels at baseline and the probability of depression onset during observation. The BDNF val66met genotype was not directly associated with onset of depression or with cortisol levels, but there was significant interaction between val66met and cortisol; the association between baseline cortisol and depression was restricted to those with the val66val genotype. It should be considered that they found a quadratic association between depressive onset and cortisol: as well as higher cortisol anticipating increased risk for MDD in their subjects, there was some evidence that levels in the lower range also represented increased risk.

In terms of remission, a number of studies have shown that the alteration of the HPA axis is an important finding in remitted MDD patients (Bhagwagar et al., 2003; Vreeburg et al., 2009). For example, Bhagwagar et al. (2003) found recovered MDD patients had a significantly greater CAR than age and gender matched comparison subjects. The waking increase in free cortisol is caused by enhanced release of ACTH, which suggests that recovered MDD patients may have abnormalities in ACTH secretion or in the responsiveness of the adrenal

gland to ACTH stimulation. That recovered patients may show abnormalities in HPA axis function, supports the argument that this abnormality may represent a trait marker of vulnerability to depression, as do the observed changes in cortisol response to the combined CRH-dexamethasone test in participants at high genetic risk of depression (Bhagwagar et al. 2003). More studies are essential to determine whether the exaggerated increase in CAR concentrations in remitted MDD patients is related prospectively to the risk of depressive relapse, or indeed to the development of comorbid medical disorders, such as coronary heart disease.

As discussed earlier, there have been inconsistencies in these findings to date. The conflicting results around the association between cortisol and MDD may have been caused by methodological variation, such as changes in salivary collection protocols, and different sample populations such as medicated or un-medicated subjects, and whether subjects were remitted, chronic or acute MDD patients. Inconsistencies may also be due to the inherent problems in reliably assessing an endocrinological system with marked diurnal as well as pulsatile variations (Lopez-Duran et al., 2009, Carnegie et al., 2013). Using a systematic and comprehensive methodological approach, and having a larger sample sizes, may lead to a more reliable of HPA axis changes in MDD (Carnegie et al., 2013). Interestingly, it has been argued that the prevalence of abnormalities is related to the measure chosen and the subtypes of depression (Young et al., 2001). Thus, when ten-minute sampling for ACTH and cortisol was performed for 24 hours in premenopausal depressed women, increased cortisol was found in MDD patients meeting criteria for endogenous (12.17 ± 4 microg/dl). There was no difference in mean cortisol between the patient groups as a whole (8.36 ± 2.9 microg/dl) and those MDD patients with atypical depression (8.38 ± 1.9 microg/dl). The baseline

component area under the curve of cortisol secretion was increased at a trend level ($P=0.64$); and the baseline AUC for ACTH was significantly increased in MDD patients.

The association between CAR as an index of HPA axis function and MDD, is still unclear. Of note, the focus on the CAR is essential, since it has been found that CAR is more reliable and useful in the studies of MDD than traditional measures of the HPA axis, such as the dexamethasone suppression test (Dedovic et al., 2015).

Beginning with subclinical depression, some studies have investigated the relationship between subclinical levels of depression and the CAR. For example, in a study of moderate and high subclinically depressed young males and females, Dedovic et al. (2010) found a flattening of cortisol level during the first hour of awakening associated with subclinical depression: lower CAR AUC_i was observed in high subclinical depression compared to the healthy controls, with moderate subclinical depression group presenting AUC_i level between these two groups (Dedovic et al., 2010). In association with this, Mangold et al. (2011) conducted a study on males and females (aged 18-38) with low, moderate, and high subclinical depression symptoms. They found that high compared to low-moderate subclinical depression symptoms were related to a flattening of the CAR. Similarly, a study investigating young males, whose scores on the Hamilton Depression Inventory were within the low, non-clinical range, showed a positive relation between low, non-clinical depression levels and the CAR AUC_i (Pruessner et al., 2003).

Focusing on clinical MDD, depression has been associated with both increased and lowered CAR in previous studies (Dedovic et al., 2015). For example, a higher CAR was observed in a large study of middle-aged MDD patients in

comparison to control subjects. Thus, Vreeburg et al. (2009) in a large cohort study showed that there is a strong link between MDD and CAR. This study consisted of three groups including control subjects without psychiatric disorders, subjects with remitted MDD, and patients with a current MDD. They observed a higher CAR among both subjects with current MDD and subjects with remitted MDD. This finding may be suggestive of an increased biological vulnerability for depression. Although both remitted and acute MDD groups had a significantly increased CAR, compared to healthy controls, surprisingly, no association was found between MDD characteristics such as severity, chronicity, symptom profile and early life stress. Similar to their findings, Ulrike et al. (2013) found a higher CAR in female MDD patients compared to controls. The CAR was calculated at 0, 30, 45, and 60 min after awakening. In line with this, in a study, consisted of women ranging in age from 17 to 23, the similar result was observed. Each participant provided salivary cortisol samples after awakening and 30 minutes after awakening on five consecutive weekdays. The main finding was that, individuals at risk for depression had higher CAR than control participants (Dienes et al., 2013). In agreement with this, Bhagwagar et al. (2005) in a study consisting of 20 acutely un-medicated MDD patients and 40 healthy controls found that although after 60 minutes of awakening the CAR was similar in healthy controls and MDD patients, there was an approximate 25% increase in the CAR in MDD patients at 30 min after awakening. The overall CAR was higher than in MDD patients than healthy controls.

Consistent with these results, studies which have focused on remitted MDD patients have generally found a higher CAR in remitted subjects. For example, higher CAR levels were found in a remitted middle-aged depressed subjects who were using medication (Vreeburg et al., 2009). In line with this finding, Bhagwagar

et al. (2003) found a similar result in remitted patients who were not using medication.

In contrast, a number of studies found a blunted or lower CAR in MDD patients compared to controls. For example, a study of young adult women with current mild to moderate clinical depression found a blunted CAR in depressed women compared to non-depressed subjects (Stetler et al., 2005). In agreement with this, older subjects without a lifetime depression and/or anxiety disorder were compared with older subjects with a duration of 6 month MDD in a study by Rhebergen et al. (2015). The MDD patients showed a decreased ability to respond to the stress of awakening among depressed older persons compared to the other group. In line with this, in a sample of outpatient MDD subjects, salivary cortisol was collected at awakening, 45min after awakening, and at 12:00 p.m., 17:00 p.m., and 21:00 p.m. Flattened diurnal patterns were associated with more severe levels of depressive episode (Hsiao et al., 2010). In agreement with these results, Miller et al. (2005) has suggested that there may be an association between a blunted CAR and moderate or mild depression in outpatients.

Some previous studies found no association between CAR and depression. For instance, Vammen et al. (2014) in a repeated cross-sectional and short-term design found that the CAR is not associated with self-reported symptoms of depression or with clinical depression. Explaining the detail of this study, a questionnaire along with salivary cortisol test tubes for home administration were sent to around 10,000 public sector employees. Morning (30min after awakening) and evening (20:00 p.m.) salivary samples were provided. They gathered around 3500 questionnaires and valid saliva samples. Approximately 4 months later, a subsample of the subjects provided three morning saliva samples (at awakening, 20min and 40min after awakening) plus an evening sample (20:00 p.m.); In line

with this, subjects with high baseline scores of self-reported depressive symptoms, burnout and perceived stress were invited to a standardized interview to diagnose clinical depression and the symptom questionnaire was repeated for subsample subjects. The study was repeated in 2 years later with questionnaires and salivary test tubes with around 2500 participants. In four cross-sectional and two short-term follow-up analyses, sex, age, income, education, family history of depression, physical activity and alcohol consumption were adjusted for subjects. In sum, they examined the impacts of several saliva cortisol measures consisted of morning, evening, mean of morning and evening but they could not find any association between cortisol levels or CAR with depressive symptoms or clinical depression.

A number of studies have focused on familial or parental history of MDD as a factor which may affect the CAR either in healthy controls with no history of depression or depressed subjects with acute MDD. It has been argued that familial risk is associated with a higher CAR even in the asymptomatic participants. Specifically, an increased CAR over the first 30 minutes after awakening in young people with high familial risk of depression compared to control participants was observed in one study (Mannie et al., 2007). They argued that this finding could not be explained by current mental state of subjects because both subjects with familial history of depression and control subjects scored the same on ratings of mood symptoms and perceived stress. They also argued that it is not clear that increased waking salivary cortisol levels can anticipate individual risk of illness and whether the increased cortisol secretion has implications for general health and cognitive function.

In another study, three groups were investigated, consisting of people without a familial history of MDD, with a self-reported familial history of MDD and with a

diagnosed familial history of MDD. These groups were compared either with each other or with a comparison group of subjects with a remitted or current major depressive disorder and/or current panic disorder with agoraphobia. They showed that, in comparison with healthy controls without a familial history of MDD, healthy subjects with familial history of depression or anxiety showed a higher CAR at 30 minutes, 45 minutes, and 60 minutes after awakening, which was similar to that observed in MDD patients with depression or anxiety disorders themselves. Unaffected people with self-reported parental history did not differ in awakening cortisol levels from unaffected people without parental history. The higher cortisol levels were only found in offspring of patients with a diagnosis treated in specialised mental healthcare and not in offspring with affected parents based on self-report. In sum, this study argued that a higher CAR reflects a trait marker, which can demonstrate an underlying biological vulnerability for the development of depressive and anxiety disorders and they demonstrated that their findings may show that self-report of parental psychopathology is of insufficient reliability (Vreeburg et al., 2010).

Findings from previous studies suggest that increased CAR could be related to MDD onset and recurrence. The work to date shows that CAR is highly heritable which represent that to a certain degree, it is a trait-like feature; however it is affected by environmental and situational items, and it may serve as an index of an individual's overall vulnerability to depression as it associates to stressful experiences. In conclusion the association between CAR and depression is complicated and future studies are essential to clarify this association (Dedovic et al., 2015).

Considering morning salivary cortisol instead of the CAR, one study conducted by Herbert et al. (2012) has suggested that there is an association between low

level of morning salivary cortisol and increased risk of MDD. Moreover, several studies have shown that cortisol level during the morning may predict the onset of major depressive episodes or symptoms (Harris et al., 2000; Halligan et al., 2007; Goodyer et al., 2010; Vrshek-Schallhorn et al., 2012). For example, in one of these studies which also focused on CAR, Vrshek-Schallhorn et al. (2012) showed that at 6 months after cortisol sampling, any one-unit rise in the baseline standardized CAR was related with a higher than 3-fold increase in risk per month for MDD onset. This risk then decays, remaining a statistically significant predictor in other sample for around 2.5 years (out of 4 years), but not significantly so after such time.

In line with this, Harris et al. (2000) has shown that morning cortisol may play a role as a risk factor for subsequent MDD in adult women. In detail, women not currently depressed, but known to be vulnerable to the onset of MDD for psychosocial reasons, were selected for baseline interview and followed up after 12 months. Their mental state was examined using the Schedule for the Clinical Assessment in Neuropsychiatry (SCAN). MDD patients were defined using DSM-IV or the Bedford College measurement. Saliva was collected over four consecutive days consisting of two times over 24 hours: at 8:00 a.m. and at 20:00 p.m. The study found an association between the subsequent onset of MDD and morning cortisol level. In particular, this was related with severe adverse life events and difficulties during the follow-up period; mean morning cortisol levels at entry; and the presence of any of three vulnerability factors.

Considering antidepressant therapy, interestingly, a number of previous studies have assessed the association between antidepressants and changes in cortisol levels (Rota et al., 2005; Nikisch et al., 2005; Aihara et al., 2007).

In particular, Rota et al. (2005) investigated the effect of Amitriptyline and Fluvoxamine on HPA axis activity in MDD patients. Plasma cortisol and ACTH levels were determined in three samples, drawn at 20min intervals between 8:00 a.m. and 9:00 a.m.; cortisol levels were determined again at 20:00 p.m. They could not demonstrate any effect of antidepressant therapy on the cortisol circadian rhythm abnormality. In another study investigating the effect of antidepressants on HPA axis function in MDD patients, a combined dexamethasone suppression and corticotropin-releasing hormone stimulation test, following a placebo week after 2, 4, and 16 weeks of 40 mg/day Citalopram treatment was performed. The study found that decreased AUC cortisol was highly associated with S-citalopram concentration in plasma. They showed that long-term, 4-6 weeks, Citalopram administration decreases the responsiveness of the HPA axis in patients with major depression.

In line with this, there are studies investigating the effect of the selective serotonin reuptake inhibitors (SSRIs) therapy on cortisol level in saliva of MDD patients, which have shown decreasing cortisol secretion as an effect of SSRI therapy (Rota et al. 2005; Nikisch et al. 2005; Mondelli et al. 2006; Aihara et al. 2007). Dziurkowska et al. (2013) observed that the level of morning salivary cortisol decreases in inpatients females after 30 days of admission and therapy.

In association with this, a randomised, blinded, placebo-controlled, parallel group design study looked at the effects of the SSRI citalopram in the first degree-relatives of patients with MDD (Knorr et al., 2012). The results of this study suggested that 4 weeks administration of Escitalopram reduces HPA axis activity in healthy controls, as the level of awakening salivary cortisol and all day salivary cortisol were lower in the subjects taking SSRI compared with subjects taking placebo.

In sum, the association of cortisol level or CAR in MDD has still remained unclear, suggesting a complex relation between cortisol and MDD.

1.4.8 The Association of the Biomarkers

It seems likely that there is an association between structural changes to the brain and other potential predictor biomarkers of MDD. For example, previous studies found a reduction of hippocampus volume in MDD patients with a history of child abuse (Heim et al., 2008). More specifically, it has been shown that the left hippocampus in MDD women with a history of early life stress is 18% smaller compared to non-abused MDD women and 15% smaller compared to healthy controls (Vythilingam et al., 2002).

Previous studies in healthy controls also provided some evidence of possible links between genetic factors, structural brain changes and early life stress. In particular, smaller amygdala and hippocampal volumes were observed in healthy met carriers with a history of childhood abuse (Gatt et al., 2008).

In agreement with a possible link between biomarkers, the association between life stress and val66met polymorphism in the BDNF gene has recently received much attention. In detail, Wichers et al. (2008), conducted a study on the study sample consisted of 621 participants aged 18-46 years who were taking part in an ongoing longitudinal, general population twin study. Childhood adversity was measured using the shortened version (Bernstein et al., 1997) of the 70-item CTQ questionnaire. Their result showed that childhood abuse had a larger impact on depression scores in BDNF met carriers than in BDNF non-met carriers. Another important finding of this study was a main effect of BDNF val/met genotype on reported childhood abuse, meaning that heterozygous val/met subjects reported significantly more childhood adversity than other subjects. Therefore, they argued that BDNF heterozygous subjects may have differences in personality that

increase the likelihood of exposure to adversities or make them more sensitive to experience stress and/or poor outcome following exposure to adversities.

In line with this, Aguilera et al. (2009) has demonstrated that childhood abuse predicted higher level of adult depressive symptoms. In detail, they found that childhood sexual abuse, childhood emotional abuse and childhood emotional neglect may play a role on symptoms of MDD. Of note, childhood sexual abuse had a stronger influence on depressive symptoms in met allele carriers of the BDNF gene in compared to the val/val group.

However, recently, a systematic review, argued that the interaction between BDNF, brain volume and childhood adversity is more convoluted, meaning that the interaction between BDNF and life stress in MDD is stronger for stressful life events compared to childhood abuse (Hosang et al., 2014).

Turning to investigations which tried to find a link between brain structural changes and endocrinological factors, Lu et al. (2013) found an association between an elevated CAR and the cingulate gyrus volume. The study comprised healthy controls with childhood abuse and age and gender matched individuals without childhood abuse. Each subject collected salivary samples in the morning at four time points: at awakening, 30, 45, and 60 min after awakening for the assessment of the CAR on the MRI scan day. All of the subjects were scanned using a 3 Tesla scanner; and VBM was conducted on structural images. The study showed an association between an increased CAR and grey matter volume reduction in the right cingulate gyrus volume in healthy controls with a history of early life stress. Of note, previous studies have found a link between early life stress and structural changes of other brain regions. In detail, increasing evidence has shown a strong link between decreased hippocampus volume and early life stress, as well as profound reductions of medial prefrontal cortex

volume. Also, it has been found that there is a correlation between deprivation and reduced orbital-fronto cortical volume in post institutionalized children (Lu et al., 2013).

In sum, it is important to consider associations between several biomarkers in MDD to make clear insight for understanding of this complex disorder; these previous studies represent first steps towards findings such associations, but future work remains to be undertaken.

1.5 Aims and Objectives

My thesis focused on the aetiology, role and interaction of genetic, neuroimaging and endocrinology measures, aiming to find a potential biomarker in MDD; it consisted of two main parts:

1. Genetics and Neuroimaging, specifically, the interaction of genetic factors and structural brain changes in MDD
2. Endocrinology, specifically, characterizing the nature of HPA axis changes in MDD, using the CAR, and investigating the effect of childhood abuse in the aetiology of HPA axis changes in MDD

1.5.1 Aims and Objectives of the first study

The aim of the first study of my thesis was to investigate the modulation of amygdala structure by the val66met BDNF gene polymorphism

In particular, the hypotheses were as follows:

1. MDD patients have a decreased amygdala volume compared to healthy controls
2. In healthy controls, met allele carriers have a decreased amygdala volume compared to the val/val genotype
3. In MDD patients, met allele carriers have a decreased amygdala volume compared to the val/val genotype

4. The effect of the met allele on amygdala volume is larger in those with MDD than in controls

1.5.2 Aims and Objectives of the second study

The main aim of the second study of my thesis was to investigate neuroendocrine abnormalities in MDD, using the CAR as an index of HPA axis, and the association of these neuroendocrine abnormalities with early life stress

The hypotheses of the second study were as follows:

1. The CAR is elevated in MDD patients compared to healthy controls
2. The CAR is elevated in those with a history of childhood abuse compared to those without such a history
3. This effect is additive in that the CAR is most elevated in those who are both depressed and have a history of ELS
4. The CAR in patients with MDD but without a history of ELS is not elevated
5. An abnormal CAR is associated with a worse clinical illness trajectory

Chapter 2 THE FIRST STUDY, METHODOLOGY AND RESULTS

2.1 Methodology

2.1.1 Subjects

Our case control study consisted of 87 recurrent MDD patients and 74 healthy controls, matched for age, sex, verbal IQ and the tendency to use either the right or the left hand.

2.1.2 Clinical Assessments

Patients had experienced two or more depressive episodes of at least moderate severity and they met criteria for recurrent MDD, as characterised by the DSM-IV using the Schedules for Clinical Assessment in Neuropsychiatry interview (Wing et al., 1990). The Schedules for Clinical Assessment in Neuropsychiatry interview was used as the main clinical assessment tool (Wing et al., 1990). This was undertaken in patients in order to confirm the presence of MDD and exclude inappropriate diagnosis, and in healthy controls to exclude a history of psychiatric disorder.

The Beck Depression Inventory was used to measure current depressive symptoms (Beck et al., 1961). The Wechsler Abbreviated Scale for Intelligence was performed to assess the IQ of participants (Wechsler, 1999). Data in regards to current use of antidepressant medications were collected from the MDD patients.

All participants had taken part in genetic association studies (Cohen-Woods et al., 2009; Uher et al., 2010).

2.1.3 Exclusion and Inclusion Criteria

Contraindications to magnetic resonance imaging (MRI) were considered for all participants during screening. Subjects with any history of head injury causing a loss of consciousness, conditions known to affect brain structure or function such

as alcohol or substance abuse, or any diagnosis of neurological disorder, were not included. If a participant, or if one of their first-degree relatives, ever fulfilled criteria for mania, hypomania, schizophrenia, or mood incongruent psychosis, they were not included.

2.1.4 Ethical Approval

Local ethics committee approval was received for the study and the subjects signed informed consent forms.

2.1.5 Neuroimaging and Genetic protocols

2.1.5.1 Genotyping

Val66met BDNF genotyping was undertaken using either polymerase chain reaction or a Taqman 5' exonuclease assay (Cohen et al., 2009). In terms of BDNF genotype, patients comprised three groups: val/val, val/met and met/met. For the purpose of the present analysis, the groups were combined into two groups – val/val homozygotes and met carriers – due to the small number of met/met homozygote subjects. Genotype frequency was tested to ensure that Hardy Weinberg equilibrium was not violated.

2.1.5.2 Neuroimaging

Magnetisation-prepared rapid gradient echo (MP-RAGE) T₁-weighted scans were acquired at 1.5 T (Signa HDx 1.5 T system, General Electric, Wisconsin, USA) with the following parameters: time to echo 3.8 ms, repetition time 8.59 ms, flip angle 8°, field of view 24 × 24 cm², slice thickness 1.2 mm, number of slices 180 and image matrix 256 × 256. The MP-RAGE volume was acquired using the Alzheimer's disease Neuroimaging Initiative (ADNI) custom pulse sequence, with full brain and skull coverage.

Grey matter volumes, surface area and average cortical thickness measurements were measured using Freesurfer version 5.1.0 (Westman et al., 2013).

Further to the priori hypothesis, analysis focussed on the amygdala as a region of interest.

A multivariate ANOVA was used to assess MDD patients and healthy controls and val/val BDNF genotypes and met carriers (val/met and met/met genotypes).

2.1.6 Statistical analysis

Data were analyzed with SPSS (Statistical Package for Social Sciences, Version 22).

A Shapiro Wilk's test, a Kolmogorov-Smirnov test and visual inspection of histograms, Q-Q plots and box plots showed that the data of our study were normally distributed, and thus parametric statistic were used.

Chi square was used to analyze categorical variables (such as gender). An independent student t test was used for comparing mean scores on continuous variables.

The prior region of interest (ROI) was the amygdala. This was analyzed for both left and right sides as a priori hypothesis.

A multivariate ANOVA was used to assess differences in amygdala volumes between healthy controls and MDD patients and between val/val BDNF genotypes and met carriers (val/met and met/met) and the estimated marginal means were presented.

If $p < 0.05$ the null hypothesis was rejected.

The hypotheses of the first study were as follows:

1. MDD patients have a decreased amygdala volume compared with controls.
2. In healthy controls, met allele carriers have a decreased amygdala volume compared with the val/val genotype.
3. In MDD patients, met allele carriers have a decreased amygdala volume compared with the val/val genotype.

4. The effect of the met allele on amygdala volume is larger in those with MDD than in controls.

Post hoc tests were undertaken using a univariate ANOVA on both right and left amygdala to find differences between: val/val and met carriers in MDD; val/val and met carriers in healthy controls; val/val MDD and val/val in controls; and met carriers in MDD and met carriers in healthy controls and the estimated marginal means were presented.

2.2 Results

Our subjects consisted of 2 groups (161 subjects) and 4 subgroups (explained at page 82)

1. Healthy Controls (74)
2. MDD Patients (87)

(Table 2.1)

Table 2.1 Number of subjects

Controls	MDD
74(46%)	87(54%)

There was no difference in terms of gender between the groups (Table 2.2).

Table 2.2 Gender

	Controls	MDD	Statistics
Male	34 (22%)	28 (17%)	p= 0.7 x ² =2.6
Female	40 (24%)	59 (37%)	

There was no difference in terms of age between groups (Table 2.3)

Table 2.3 Age

	Controls	MDD	Statistics
Age (years)	50.9±7.8	48.5±9.1	p= 0.08 t=1.7

There was no difference in terms of Verbal IQ between groups (Table 2.4).

Table 2.4 Verbal IQ

	Controls	MDD	Statistics
Verbal IQ	119.0±8.7	116.8±11.4	p=0.1 t=1.3

There was no difference in terms of the tendency to use either the right or the left hand between groups (Table 2.5).

Table 2.5 The tendency to use either the right or the left hand

	Controls			MDD			Statistics
	Right	Left	Right and Left	Right	Left	Right and Left	
Hands	63(86%)	9 (12%)	1(2%)	74(84%)	11(12.5%)	3 (3.5%)	p=0.6 x ² = 0.7

There was no difference in terms of BDNF genotype between groups (Table 2.6).

Table 2.6 Genotype

	Controls		MDD		Statistics
	val/val	met carriers	val/val	met carriers	p=0.6 x ² = 0.1
BDNF	47 (63.5%)	27 (36.5%)	52 (59%)	35(41%)	

2.2.1 Comparing the amygdala volume between MDD patients and healthy controls

Although the mean amygdala volume was numerically lower in MDD patients, there was no significant volume reduction in MDD patients compared with controls (right amygdala-p=0.09; left amygdala-p=0.1) (Table 2.7)

Table 2.7 Comparing the amygdala volume between MDD patients and healthy controls

Brain Region	MDD (Mean Volume) mm ³	SD	Controls (Mean Volume) mm ³	SD	p
Right Amygdala	1479.1	230.5	1552.5	271.3	0.09
Left Amygdala	1377.1	220.3	1433.8	257.1	0.1

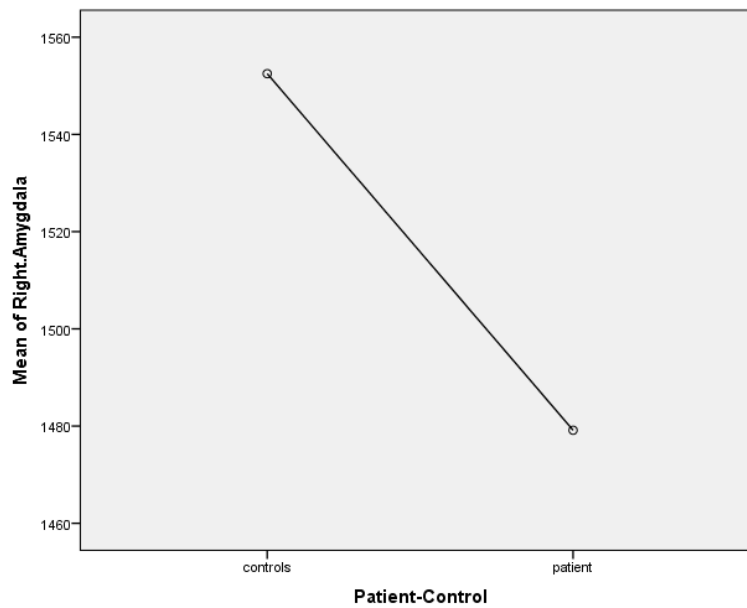


Figure 2-1 Right amygdala volume in MDD patients and healthy controls

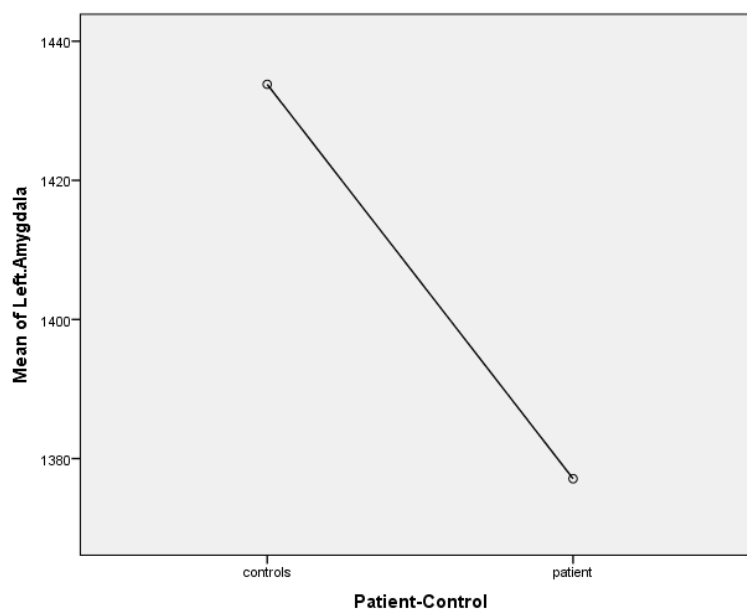


Figure 2-2 Left amygdala volume in MDD patients and healthy controls

2.2.2 Comparing the amygdala volume between Val/Val Carriers and Met Carriers (Val/Met, Met/Met)

Although the amygdala volume was smaller in met carriers compared with the val/val BDNF genotype, the reduction was not significant (right amygdala- $p=0.3$; left amygdala- $p=0.1$) (Table 2.8)

Table 2.8 Comparing the amygdala volume between val/val carriers and met carriers

Brain Region	met carriers mm ³	SD	val/val BDNF genotype mm ³	SD	p
Right Amygdala	1486.8	252.5	1529.1	251.5	0.3
Left Amygdala	1372.2	216.02	1422.5	251.2	0.1

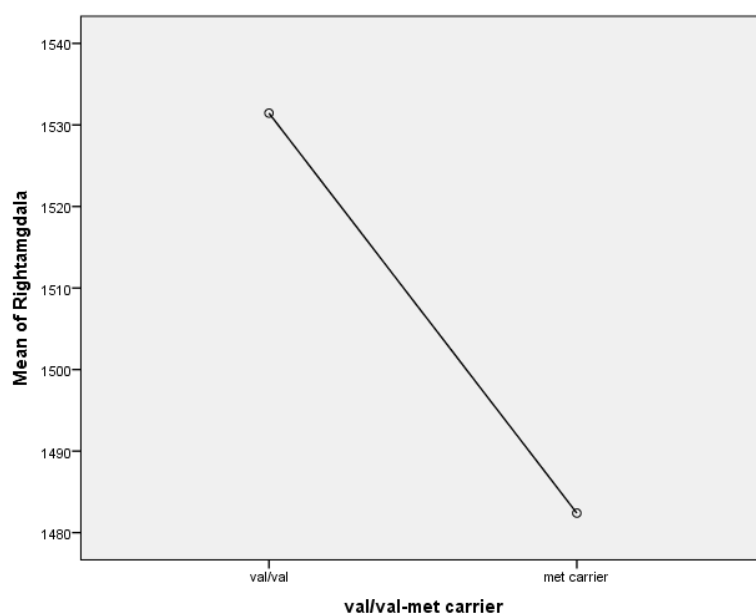


Figure 2-3 Comparing the right amygdala volume between val/val carriers and met carriers

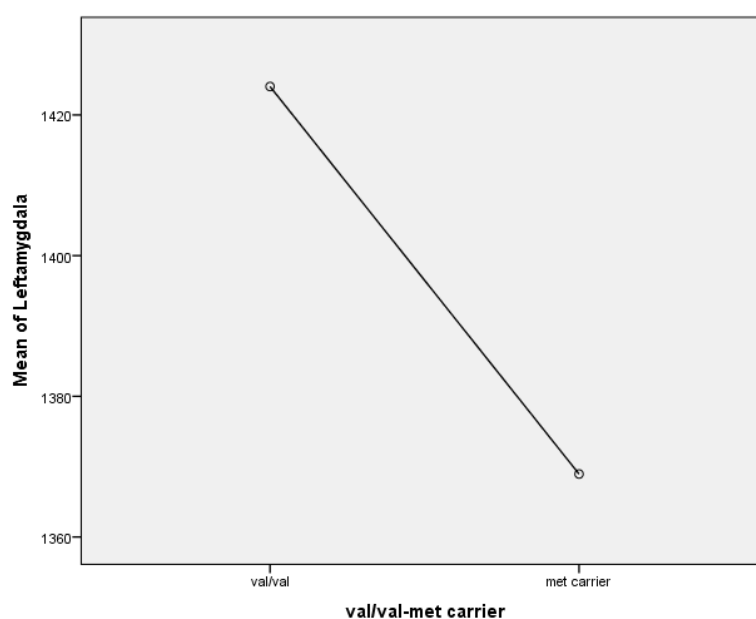


Figure 2-4 Comparing the left amygdala volume between val/val carriers and met carriers

There was no significant interaction between BDNF genotype and groups—or sub groups explained on page 82—on amygdala volume either in right or left amygdala (right amygdala- $p=0.06$; left amygdala- $p=0.2$). The estimated marginal mean values represent the mean value for each factor, adjusted for any other variables in the model. - The estimated marginal means of right and left amygdala in MDD patients and controls are shown in the figures below (2.5, and 2.6)

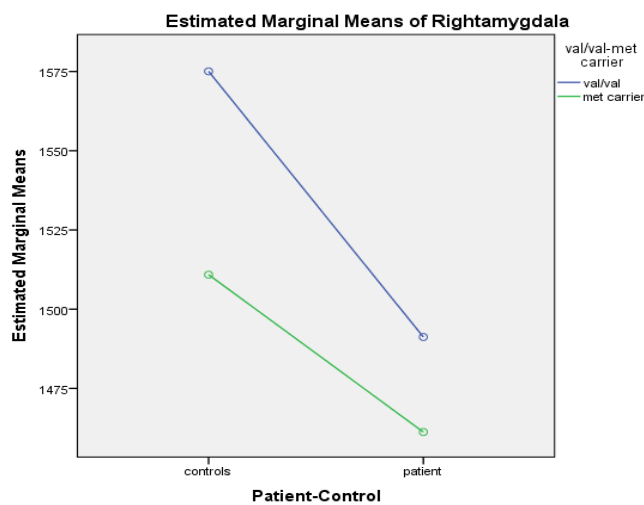
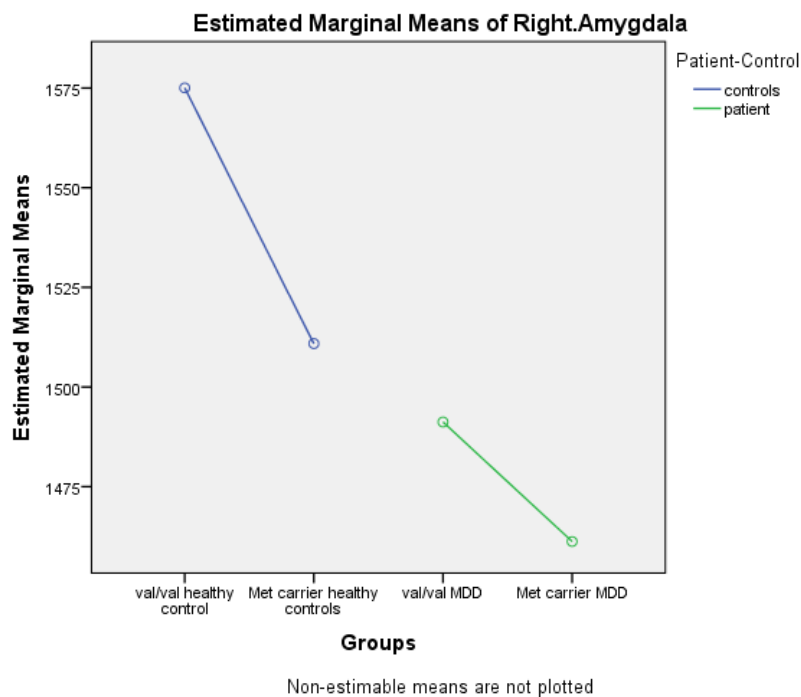


Figure 2-5 No significant interaction between BDNF genotype and groups on right and left amygdala volumes

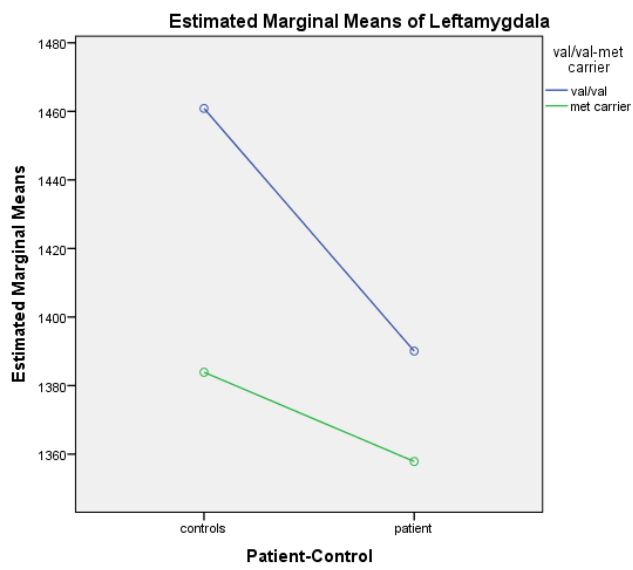
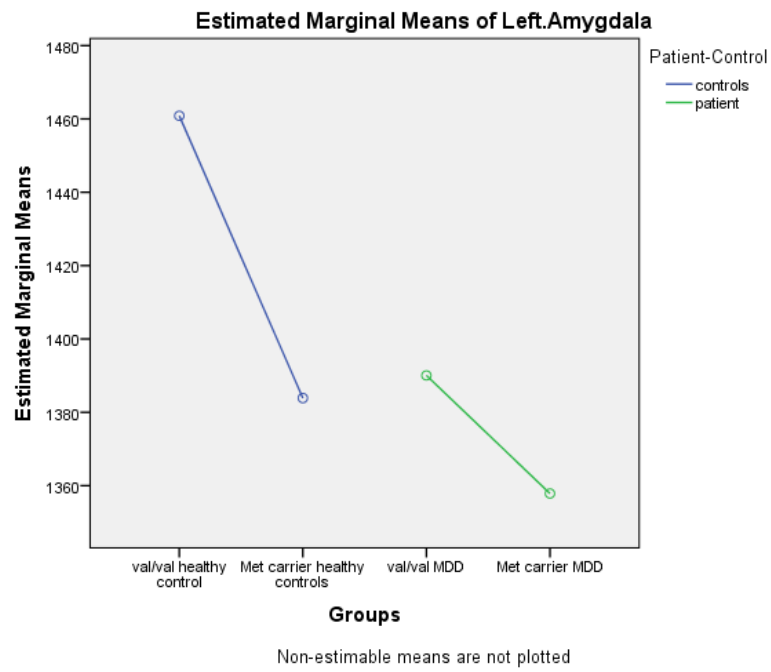


Figure 2-6 No significant interaction between BDNF genotype and groups on left amygdala volume

In order to test our hypotheses, new sub groups were then formed, consisting of healthy controls and MDD patients as below (Table 2.9)

1. val/val BDNF healthy controls (HC): 48 subjects
2. met carriers healthy controls (HC): 26 subjects
3. val/val BDNF MDD : 52 subjects
4. met carriers MDD: 35 subjects

Table 2.9 New groups in regards to genotype

val/val HC	met HC	val/val MDD	met MDD
48(30%)	26(16%)	52(32%)	35(22%)

2.2.3 Comparing the amygdala volume between Met Carriers and Val/Val Carriers in healthy controls

The amygdala volume was numerically smaller in met carriers compared with val/val homozygotes in healthy controls, but the reduction was not statistically significant (right amygdala- $p=0.7$; left amygdala- $p=0.5$). Table 2.10

Table 2.10 Comparing the amygdala volume between met carriers and val/val carriers in healthy controls

Brain Region	val/val Mean Volume mm ³	SD	met carrier Mean Volume mm ³	SD	p
Healthy Controls					
Right Amygdala	1575.0	263.1	1510.8	286.2	0.7
Left Amygdala	1460.8	267.8	1383.8	232.9	0.5

2.2.4 Comparing the amygdala volume between Met Carriers and Val/Val Carriers in MDD patients

The amygdala volume was numerically smaller in met carriers compared with val/val homozygotes in MDD patients, but the reduction was not statistically significant (right amygdala- $p=0.9$; left amygdala- $p=0.9$) (Table 2.11).

Table 2.11 Comparing the amygdala volume between met carriers and val/val carriers in MDD patients

Brain Region	val/val Mean Volume mm ³	SD	met carrier Mean Volume mm ³	SD	P
Right Amygdala	1491.2	235.2	1461.2	225.5	0.9
Left Amygdala	1390.06	230.7	1357.8	205.6	0.9

2.2.5 Comparing the amygdala volume between Met Carriers in healthy controls and Met Carriers in MDD patients

There was no statistically significant difference in amygdala volume between met carriers in healthy controls and met carriers in patients. Patients had a numerically smaller amygdala volume which was not significant (right amygdala- $p=0.8$; left amygdala- $p=0.9$) Table 2.12

Table 2.12 Comparing the amygdala volume between met carriers in healthy controls and met carriers in MDD patients

Brain Region	met carriers Mean Volume HC mm ³	SD	met carrier Mean Volume Patients mm ³	SD	p
Right Amygdala	1510.8	286.2	1461.2	225.5	0.8
Left Amygdala	1383.8	232.9	1357.8	205.6	0.9

2.2.6 Comparing the amygdala volume between Val/Val Carriers in healthy controls and Val/Val Carriers in MDD patients

The amygdala volume was numerically smaller in MDD patients compared with healthy controls, but the reduction was not statistically significant (right amygdala- $p=0.3$; left amygdala- $p=0.4$) Table 2.13

Table 2.13 Comparing the amygdala volume between val/val carriers in healthy controls and val/val carriers in MDD patients

Brain Region	val/val Mean Volume HC mm ³	SD	val/val Mean Volume Patients mm ³	SD	Stat
Healthy Controls and Patients					
Right Amygdala	1575.0	263.1	1491.2	235.2	$p=0.3$ $F=1.6$
Left Amygdala	1460.8	267.8	1390.0	230.7	$p=0.4$ $F=1.4$

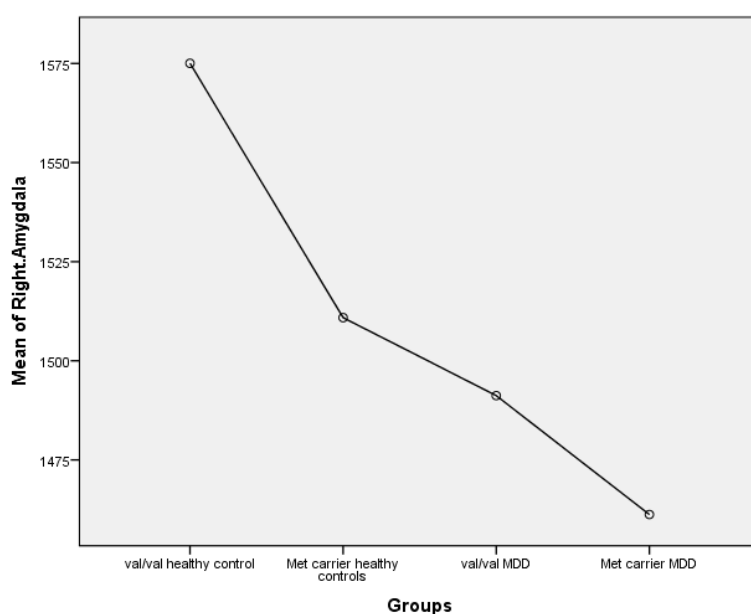


Figure 2-7 Comparing the right amygdala volume between groups

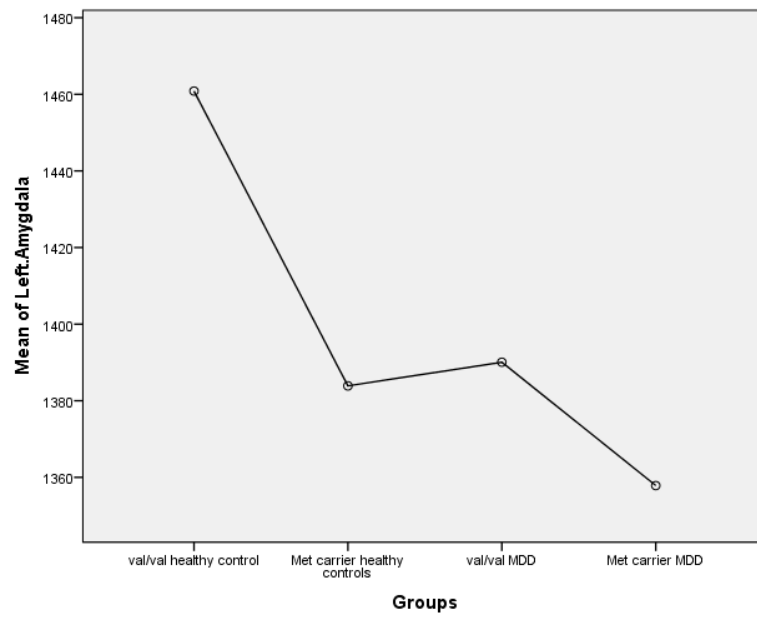


Figure 2-8 Comparing the left amygdala volume between Groups

Chapter 3 THE SECOND STUDY, METHODOLOGY AND RESULTS

3.1 Methodology

The study used a cross-sectional design with four subject groups, consisting of healthy controls with no diagnosis of any psychiatric disorder and no history of childhood trauma (CB), healthy controls with no diagnosis of psychiatric disorder but with a history of childhood trauma (CA), MDD patients without a history of childhood trauma (PB), and MDD patients with a history of childhood trauma (PA).

3.1.1 Subjects

Subjects aged 18 to 75 years who fulfilled the inclusion criteria for one of the four study groups were recruited into the study.

MDD patients were recruited from inpatients and outpatients of the Affective Disorders Service.

All patients had a history of unipolar MDD diagnosed according DSM-IV. MDD patients who had an organic cause for their depression, had any illness or neurological problem that might affect the HPA axis, were current heavy smokers (more than 40 cigarettes in 24 hours); were regularly using corticosteroid, had alcohol or drug dependency or who were pregnant or nursing were not included in the study.

Healthy controls were recruited from the general population, including a number of healthy controls who were part of the volunteer database and had previously taken part in other studies of the Affective Disorders Research Group.

Healthy controls who had a problem that could affect the HPA axis, such as a personal history of psychiatric illness as defined by DSM-IV, psychiatric illness in any first-degree relative, use of medication which could affect the HPA axis,

pregnancy or lactation, endocrinological disorders and heavy smoking (more than 40 cigarettes in 24 hours) were not included.

Subjects were assessed either at the Inpatient Affective Disorders Unit – for those who were inpatients at the time of assessment – or at the subjects' home. For those undertaking the study at home, we posted a package consisting of instructions on how to collect saliva in saliva tubes, questionnaires and an envelope in order to return the package to the laboratory. Further support and instructions were delivered to them by telephone. All procedures were approved by the hospital ethics committee. Both MDD patients and healthy controls gave written informed consent form prior to participating to the study.

3.1.2 Clinical Assessments

Demographic and clinical information was collected using the following forms and questionnaires.

3.1.2.1 International Classification of Diseases - ICD-10 Criteria

MDD patients underwent full clinical assessment by a psychiatrist and were required to meet both DSM-IV and ICD-10 criteria for major depressive disorder or major depressive episode.

3.1.2.2 Hamilton depression rating scale 17 items and 21 items

For assessing the clinical severity of MDD, all subjects were interviewed using the Hamilton Depression rating scale 17 items (HAM-D 17) and 21 items (HAM-D 21) versions (Hamilton, 1960). We used Hamilton 21 items to provide more information such as whether diurnal variation or paranoid symptoms are present.

3.1.2.3 Clinical History

Psychiatric and clinical history was obtained from MDD patients, including duration of current depressive episode, age of onset of first depressive episode,

total duration of illness, the number of previous depressive episodes and the number of previous antidepressants used.

3.1.2.4 Stage of Treatment Resistance

The Maudsley Staging Method was applied to measure the degree of treatment resistance in MDD patients (Fekadu et al., 2009b). They were assessed by psychiatrists and the patients consisted of mild, moderate and severe stage.

3.1.2.5 Self-Rated Questionnaires

Questionnaires were sent to the subjects for measuring the severity of depressive symptoms, anxiety, current level of health and function, disability, life events over the last 12 months, symptoms of PTSD, sleep quality, current social stress and early life stress (childhood trauma). They were completed by the subjects and returned with the saliva packs.

This process led to some questionnaires being incomplete or missing. Where possible missing data were then collected by telephone; where appropriate and available, data from previous studies they may have taken part in were used.

Total scores were summed for each questionnaire and the correlation between the CAR and each questionnaire was calculated in all groups. If the data were normally distributed, one way ANOVA – or t test if there was data only for two groups – was used to compare between groups. If the data were not normally distributed, Mann-Whitney U test or Kruskal-Wallis test was conducted.

3.1.2.5.1 IDS30 - Inventory of Depressive Symptomatology (IDS) (Rush et al., 1986)

The Inventory of Depressive Symptomatology (IDS) is a questionnaire used for measuring signs and symptoms of MDD. There are many versions of this questionnaire; for this study we used the 30-item self-report version. It is a reliable questionnaire and shows good correlation with both the HAM-D and the BDI. The

factor structure for the IDS30 shows four factors: mood/cognition, anxiety, selected endogenous symptoms, and hyperphagia-hypersomnia. The IDS appears suitable for both inpatients and outpatients with endogenous, atypical, and non-endogenous MDD.

3.1.2.5.2 Zung Self-rating Anxiety Scale (Zung, 1971)

The Zung Self-rating Anxiety Scale quantifies symptoms of anxiety and is simple for subjects to fill in. It is available in two formats, for participant and observer. In this study the self-report version was used consisting of 20 questions. The subjects are asked to mark the correct column for each symptom as below (Table 3.1).

Table 3.1 ZSAS

Little of the time	Some of the time	Good part of the time	Most part of the time

3.1.2.5.3 Medical Outcomes Survey Short Form 36 (SF-36)

The SF-36 is a short-form and multi-purpose health survey with 36 questions. It consists of 8-subscales profiling functional health and well-being scores; psychometrically-based physical and mental health measures; and a preference-based health utility index. It is a general measure of function and quality of life. Of note, the SF-36 has been widely used both in the general population and in specific subject groups to compare the burden of illness, and to clarify the health benefits produced by many types of treatments.

The SF-36 was designed to satisfy minimum psychometric standards necessary for group comparisons. The eight health concepts were chosen from 40 included in the Medical Outcomes Study (MOS) (Ware et al., 1992). Those chosen indicate the most frequently measured concepts in broadly-used health surveys and those most affected by illness and of note treatment (Ware et al., 1993; 1995). The questionnaire items represent multiple operational indicators of health, including:

behavioral function and dysfunction; distress and well-being, objective reports and subjective ratings, and both favorable and unfavorable self-evaluations of general health status (Ware et al., 1993).

3.1.2.5.4 Work and Social Adjustment Scale (WSAS)

The Work and Social Adjustment Scale (WSAS) is a simple, widely-used 5-item measure of disability.

The maximum score on the WSAS is 40, lower scores being better. A WSAS score above 20 appears to suggest moderately severe or worse psychopathology. Scores between 10 and 20 are associated with significant functional impairment but less severe clinical symptomatology. Scores below 10 appear to be associated with subclinical populations. Whether such a pattern will generalize to other illness (apart from OCD and depression) remains to be tested. The WSAS is a valid and change-sensitive measure of work/social and other adjustment in phobia, in particular in agoraphobia and social phobia.

3.1.2.5.5 OSLO Social Support Scale (OSS-3)

The OSS-3 provides a brief measure of social functioning and is recognized to be one of the best predictors of future mental health problems. It assesses several aspects of social support by measuring the number of people the participant feels close to, the interest and concerns shown by others, and the ease of gaining practical help from the others. The OSS-3 scoring system is from 3-14, with a score of 3-8 indicating poor support, 9-11 moderate support and 12-14 strong support.

3.1.2.5.6 List of Threatening Events (LTE)

The List of Threatening Events was developed from a larger scale devised by Brugha et al. (1985) that had 9 more items. The scale's purpose is to quantify the

occurrence of recent life events, focusing on those happening in the last 12 months and whether the subject thinks they are having a continuing effect.

3.1.2.5.7 Impact of Events Scale (IES)

This scale is used to measure the impact that a trauma has had on a person and can be used to quantify severity of PTSD symptoms. This instrument measures the ongoing degree of stress caused by traumatic events, centered on two subscales: Intrusion and Avoidance.

The format of the scale is a 15-item self-report in which subjects identify a stressful event and then reply to subsequent questions referring to the effects of this event using a 4-point scale.

3.1.2.5.8 Pittsburgh Sleep Quality Inventory (PSQI)

This is a self-related questionnaire comprised of seven components each rated weighted from 0 to 3 resulting in a global score of 0-21. The higher the overall score, the worse the quality of sleep. There are 19 items in the main questionnaire and 5 questions for the bed partner that are just for clinical information and not for scoring. It is easy for subjects to fill in and it usually takes 5 minutes. The scale has high sensitivity and specificity for insomnia patients compared to the healthy controls. The components assessed by the scale include duration of sleep, latency of sleep, and quality of sleep, sleep interruption and use of medication.

3.1.2.5.9 Perceived Stress Scale (PSS)

This is the most extensively used psychological instrument for assessing individuals' current perceptions of the level of subjective stress they are experiencing. It is a measure of the degree to which situations are assessed as stressful in one's life, and includes elements to assess how uncontrollable, and overwhelmed, subjects find their lives. The subjects are asked how often they felt or thought in a certain way over the past month. The questions are

comprehensive in nature and free of content specific to any sub population group. All items begin with the phrase: “In the past month, how often have you felt...?” The 10-item of this self-report instrument with a five-point scale was used (Table 3.2).

Table 3.2 PSS

0 = never, 1 = almost never, 2 =sometimes, 3 = fairly often, 4 = very often

3.1.2.5.10 Childhood Trauma Questionnaire (CTQ) (Bernstein, 1998)

The presence or absence of childhood trauma was determined using the Childhood Trauma Questionnaire (Bernstein et al., 1998).

The CTQ is a 28-item, self-report instrument that includes five subscales (emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect). Published guidelines for the classification of CTQ subscale total scores were applied to determine threshold severity or absence of maltreatment on each subscale. It is an effective, clinical tool, which can be administered in 5 minutes. Subjects generating scores for low/minimal levels of maltreatment were not chosen for the abused groups in the study (Bernstein et al., 2003; Carpenter et al., 2007).

3.1.3 Cortisol Awakening Response (CAR)

3.1.3.1 Saliva Sample Collection

Subjects were asked to collect six saliva samples in plain polypropylene tubes by using the drooling method. Plastic straws were available to aid with the collection. The test was undertaken on any normal weekday except Monday, with appropriate precautions taken for those working irregular patterns to avoid testing on the morning after a rest day. Women were asked to collect the samples during the first ten days of their menstrual cycle, i.e. during the follicular stage. Subjects were guided by careful instructions to collect six samples of saliva over the course

of a morning. The first one should have been collected at awakening and the other samples at 15, 30, 45, 60 and 90 minutes after the first one.

Although the actual time of collecting was not controlled, subjects were asked to take the first sample while they were relaxed and still in bed. Subjects received a full set of tubes and details of collection. They were informed about not brushing their teeth and not eating or drinking at least one hour before collection. This was in order to avoid falsely high cortisol values due to plasma exudates, or from meal-stimulated rises in cortisol. They were instructed to avoid stressful situations and try to remain relaxed during the collection. In order to control for the time that the subjects gave the samples and to ensure that other confounders for the CAR were not missed, subjects completed a form for the day they collected the salivary samples. Subjects were instructed to fill in the time of sample collection and to comment on their activities and mood state during the collection. Subjects were instructed to add in the forms whether they experienced any form of physical pain or any other information that they could think would be of relevance and that could be problematic for the study and the protocol. They were informed to write about any unavoidable stressful events during collection.

Subjects were advised to use the parafilm which was in the pack they received, if they experienced a dry mouth. They were asked to keep the samples in a fridge and then send them back to the lab. After arriving at the laboratory, samples were kept at -20°C in the freezer until analysis.

Subjects who collected the samples at home were provided with a prepaid envelope in order to post the samples back to the Affective Disorders Unit.

3.1.3.2 Salivary Cortisol Analysis

Saliva cortisol concentrations were determined using the chemiluminescence assay on the 'Immulite'-DPC's automated Immunoassay analyser (www.diagnostic.siemens.com).

The method correlated well with an in-house and previously published (Jurueña et al., 2006) Time-Resolved Fluoroimmunoassay (TR-FIA) ($r=0.94$, $y=0.004+1.08x$, $N=41$). It had analytical sensitivity of 0.2 nm/l, mean recovery of cortisol of 106.1% (range from 5 to 65 nm/l) and inter/intra-assay precision (% CV) of less than 10% (range 5 to 25 nm/l).

The linearity upon dilution test (parallelism) resulted in a straight line ($r=0.99$, $y=0.144+1.014x$). The calibration graph was highly reproducible ($n=11$ assays) with slope (mean \pm sem) of 0.197 ± 0.004 .

3.1.3.3 Area Under the Curve (AUC)

The methodology for calculating the CAR was described earlier in my thesis (Section 1.3.5.6). Further details are as below.

AUC_G and AUC_I were measured and calculated between 0 and 90 min as described earlier using the methods of Pruessner et al. (2003). The precise formulas used for the calculation of AUC were as follows:

$$AUC_G = \sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2}$$

With t_i denoting the individual time distance between measurements, m_i the individual measurement, and n the total number of measures.

$$AUC_I = \left(\sum_{i=1}^{n-1} \frac{(m_{(i+1)} + m_i) \cdot t_i}{2} \right) - \left(m_1 \cdot \sum_{i=1}^{n-1} t_i \right)$$

With m_i denoting the single measurements, t_i denoting the time distance between the measurements, and n denoting the total number of measurements.

Applying these formulae to the data in this study gave the following calculations:

$$\text{AUCg} = (\text{cortisol1} + \text{cortisol0})/2 \times 15 + (\text{cortisol2} + \text{cortisol1})/2 \times 15 + (\text{cortisol3} + \text{cortisol2})/2 \times 15 + (\text{cortisol4} + \text{cortisol3})/2 \times 15 + (\text{cortisol5} + \text{cortisol4})/2 \times 30$$

$$\text{AUCi} = ((\text{cortisol1} + \text{cortisol0})/2 \times 15 + (\text{cortisol2} + \text{cortisol1})/2 \times 15 + (\text{cortisol3} + \text{cortisol2})/2 \times 15 + (\text{cortisol4} + \text{cortisol3})/2 \times 15 + (\text{cortisol5} + \text{cortisol4})/2 \times 30) - (\text{cortisol0} \times 90)$$

Where cortisol 0 is the value at 0 minutes post awakening, cortisol1 is the value at 15 minutes post awakening, cortisol2 is the value at 30 minutes post awakening, cortisol3 is the value at 45 minutes post awakening, cortisol 4 is the value at 60 minutes post awakening and cortisol 5 is the value at 90 minutes post awakening.

If the cortisol values at 0 minutes and/or 90 minutes were missing data, the AUC was not measured. For the cases having other missing values – i.e. not at 0 or 90 minutes – the mean value of the two proximal measures was used and the value interpolated.

3.1.4 Statistical Analysis

The study was powered to detect an effect size of 0.4, with 20 patients in each group and setting $\alpha = 0.05$ at a power of 0.9.

Data were analyzed with SPSS (Statistical Package for Social Sciences) Version 22.

A value of $p < 0.05$ was considered as statistically significant (if $p < 0.05$ the null hypothesis was rejected). All p values reported were two tailed.

A Shapiro Wilk's test, a Kolmogorov-Smirnov and visual inspection of their histograms, Q-Q plots and box plots were used to determine if the data were normally distributed. Chi square was used to analyze categorical variables such

as gender. An independent student t test was presented for comparing the mean score on the continuous variables if the data were normally distributed.

If the data were not normally distributed, data were transformed using a natural log transformation prior to analysis.

In our analysis, the data were not normally distributed, and log transformation could not be used (e.g. there were negative values), or did not lead to a normal distribution. Therefore non-parametric tests were used. In this case, Mann-Whitney U test for analysis of two independent groups on continuous measures and Kruskal-Wallis test to test the scores on continuous variables for four groups were undertaken. In the tables of our analysis, we reported all median, IQR, mean and standard deviations.

We used boxplots to find the outliers in our analysis. An outlier is an observation that lies an abnormal distance from other values in a random sample from a population. The box plot is a standardized way of displaying the distribution of data based on the five sample statistics: minimum, first quartile, median, third quartile, and maximum. In box plot, the central rectangle spans the first quartile to the third quartile (the interquartile range or IQR). A segment inside the rectangle presents the median and "whiskers" above and below the box show the locations of the minimum and maximum. There are two types of outliers:

1. Extreme outliers are either $3 \times \text{IQR}$ or more above the third quartile or $3 \times \text{IQR}$ or more below the first quartile.
2. Suspected outliers are slightly more central versions of outliers: either $1.5 \times \text{IQR}$ or more above the third quartile or $1.5 \times \text{IQR}$ or more below the first quartile.

In all cases of outliers, we looked back at the data collection sheets to find any probable error in collection that might account for the abnormal values. After that we checked all the forms to find the possible cause of having outliers in our data.

We did not include any outliers who had cortisol values of more than 25 nm/l at all time points.

The AUC was defined as the main outcome variable. Both AUCg and AUCi were used.

Correlation analysis between the AUC and cortisol values at all individual points on one hand, and psychometric measures on the other, was performed using Spearman correlation coefficients, giving values from 1 to -1 for positive correlations or negative correlations respectively.

3.2 Results

A total of 140 subjects were recruited from the Affective Disorders Unit and the healthy population as described previously.

Of these, 10 subjects were subsequently excluded as they were in the remission phase of depression (HAM-D ≤ 7 if HAM-D-17; ≤ 8 if HAM-D-21) at the time of testing, 10 subjects were not included because their depression was of subclinical intensity (HAM-D 8-13 on the HAM-D-17; or 9-15 on the HAM-D-21). A further 5 subjects were not included due to protocol violations in the cortisol collection, either because they did not collect cortisol 0 – the cortisol sample at the time of awakening – or cortisol 5 – the cortisol sample at 90 minutes; or because they had 2 missing values at other time points preventing interpolation of missing data. One subject was not included because of failure to do sample collection within the appropriate time (collecting over 4 hours instead of 90 minutes). One subject was not included because the level of cortisol was more than 25 nm/l at all the time points. Finally, one subject refused to sign the consent form.

The final included sample was 112 subjects divided into 4 groups (Table 3.3)

1. Group 1: Healthy controls without a history of childhood trauma (CB, controls non-abused)

2. Group 2: Healthy controls with a history of childhood trauma (CA, controls abused)
3. Group 3: MDD patients without a history of childhood trauma (PB, depressed non-abused)
4. Group 4 :MDD patients with a history of childhood trauma (PA, depressed-abused)

Table 3.3 Number of subjects in all groups

CB	CA	PB	PA
43 (38.4%)	26(23.2%)	15(13.4%)	28(25%)
Healthy Controls (CB and CA)		Patients (PB and PA)	
69 (61.6%)		43 (38.4%)	
Non-Abused (CB and PB)		Abused (CA and PA)	
56 (50%)		56(50%)	

3.2.1 Hamilton depression rating scale scores

There was no difference between CB and CA in terms of HAM-D 17 ($p=0.9$, $z=-0.2$) and HAM-D 21 ($p=0.7$, $z=-0.3$). There was no difference between PB and PA in terms of HAM-D 17 ($p=0.06$, $z=-1.8$) and HAM-D 21 ($p=0.06$, $z=-1.8$). Table 3.4

Table 3.4 Hamilton Depression Rating Scale scores

		CB	CA	Statistics	PB	PA	Statistics
HAM-D 17	Median,(IQR)	0,(2)	0.5,(3)	$P=0.9$	18,(4)	23.5,(9)	$P=0.06$
	Mean \pm SD	1 \pm 1.4	1.2 \pm 1.3	$Z=-0.2$	20.4 \pm 5.9	23.3 \pm 5.1	$Z=-1.8$
HAM-D 21	Median,(IQR)	0,(2)	0.5,(3)	$P=0.7$	19,(6)	24.5,(10)	$P=0.06$
	Mean \pm SD	1 \pm 1.4	1.3 \pm 1.4	$Z=-0.3$	20.7 \pm 5.9	23.7 \pm 5.3	$Z=-1.8$

3.2.2 Comorbidity

Among patients, there was one non-abused MDD patient with OCD (obsessive compulsive disorder) and GAD (Generalized Anxiety Disorder), one non-abused MDD patient with OCD and Tick Disorder, one non-abused MDD patient with

OCPD (Obsessive Compulsive Personality Disorder), one non-abused MDD patient with GAD and one abused MDD patient with GAD.

3.2.3 Psychosis

Two non-abused MDD patients and two abused MDD patients suffered with depression with psychotic symptoms.

3.2.4 Duration of MDD

The median duration of MDD since the age of onset was 16 (IQR=24) years in PB group, and 17 (IQR=26) years in the PA group. There was no difference between the two groups (11 PB, 12PA) ($p=0.8$, $z=-0.1$). Table 3.5

Table 3.5 Duration of MDD

Group	Duration of Illness (year)		Statistics
	Median,(IQR)	Mean \pm SD	
PB	16,(24)	21.1 \pm 15.5	$p=0.8$ $z=-0.1$
PA	17,(26)	21 \pm 16.9	

3.2.5 Age of onset of the first episode

The median age of onset of first episode was 25 (IQR=16) for non-abused MDD patients and 29 (IQR=16) for abused MDD patients. There was no difference between the two groups (12PB, 15PA), ($p=0.2$, $z=-1.1$). Table 3.6

Table 3.6 Age of onset of first episode

Group	Age of onset of first episode		Statistics
	Median,(IQR)	Mean \pm SD	
PB	25,(16)	27 \pm 9.7	$p=0.2$, $z=-1.1$
PA	29,(16)	31.4 \pm 10.8	

3.2.6 Duration of the current episode

The median duration of current episode was 3 (IQR=3) years in the non-abused MDD patients, and 3.5 (IQR=8.5) years in the abused MDD patients. There was no difference between the groups (PB=11, PA=12) ($p=0.8$, $z=-0.1$) Table 3.7

Table 3.7 Duration of current episode

Group	Duration of current episode(years)		Statistics
	Median,(IQR)	Mean \pm SD	
PB	3,(3)	4.2 \pm 2.7	$p=0.8$, $z=-0.1$
PA	3.5,(8.5)	7 \pm 8.2	

3.2.7 Number of previous episodes

There was no difference in median numbers of previous episodes between patient groups. The median number of episodes was 1 (IQR=2) for non-abused patient and 4 (IQR=5) for abused patients (PB=11, PA=15) ($p=0.1$, $z=-1.6$) Table of 3.8

Table 3.8 Number of previous episodes

Group	Number of previous episodes		Statistics
	Median,(IQR)	Mean \pm SD	
PB	1,(2)	1.3 \pm 1.7	$p=0.1$ $z=-1.6$
PA	4,(5)	4 \pm 4.2	

3.2.8 Number of previous antidepressants

MDD patients were taking several types of antidepressants. The median number of previous antidepressants in non-abused patients was 5.5 (IQR=5); although use of antidepressants was numerically higher in abused patient 8.5 (IQR=5) there was no significant difference between two groups (PB=12, PA=14)

($p=0.1$, $z=-1.6$) Table 3.9

Table 3.9 Number of previous antidepressants

Group	Number of previous antidepressants		Statistics
	Mean,(IQR)	Median \pm SD	
PB	5.5,(5)	6 \pm 4.1	$p=0.1$ $z=-1.6$
PA	8.5,(5)	8.2 \pm 3.5	

There was no significant correlation between the cortisol at any individual time points or between AUC and duration of current episode, age of onset of first episode, duration of MDD, number of previous depressive episodes and number of previous antidepressants. The only significant positive correlation was found between cortisol₁₅ and age of onset of first episode, such that when the first episode occurs later in life the cortisol is higher at 15 minutes after awakening ($p=0.04$, $r=0.3$) Table 3.10

Table 3.10 Correlations between cortisol, AUC, and clinical characteristics

	Cortisol0	Cortisol15	Cortisol30	Cortisol45	Cortisol60	Cortisol90		
current episode	p=0.9	p=0.7	p=0.8	p=0.9	p=0.8	p=0.8		
	r=-0.02	r=-0.06	r=-0.04	r=-0.01	r=-0.05	r=-0.04		
first episode	p=0.08	p=0.04	p=0.08	p=0.5	p=0.3	p=0.1		
	r=0.3	r=0.3	r=0.3	r= 0.1	r= 0.1	r=0.2		
duration	p=0.4	p=0.6	p=0.5	p=0.4	p=0.5	p=0.6		
	r= 0.1	r=0.1	r=0.1	r=0.1	r=0.1	r=0.1		
previous episode	p=0.3	p=0.3	p=0.7	p=0.7	p=0.3	p=0.1		
	r=0.1	r= 0.1	r=0.06	r=-0.06	r=-0.2	r =-0. 3		
previous antidepressant	p=0.9	p=0.8	p=0.4	p=0.1	p=0.4	p=0.1		
	r=-0.07	r=-0.04	r=0.01	r=0.2	r=0.1	r=0.3		
	AUCg60		AUCi60		AUCg90		AUCi90	
current episode	p=0.9		p=0.8		p=0.9		p=0.8	
	r=0.01		r=0.04		r=0.009		r=0.04	
first episode	p=0.1		p=0.7		p=0.1		p=0.6	
	r=0.2		r=0.06		r=0.2		r=0.09	
duration	p=0.5		p=0.8		p=0.4		p=0.6	
	r=0.1		r=0.04		r=0.1		r=0.1	
previous episode	p=0.9		p=0.4		p=0.6		p=0.3	
	r=0.006		r=-0.1		r=-0.09		r=-0.1	
previous antidepressant	p=0.3		p=0.2		p=0.3		p=0.3	
	r=0.1		r=0.2		r=0.2		r=0.1	

3.2.9 Gender

MDD patients and healthy controls were not similar in terms of gender, with a higher number of females compared to males in the patient group ($p=0.009$; Table 3.11). There was no difference between the abused and non-abused groups ($p=0.5$). There was also a significant difference among the 4 groups on

Chi square testing when considered separately ($p=0.03$), but this appeared to reflect the gender difference noted below Table 3.11

Table 3.11 Gender

		CB	CA	PB	PA	Statistics
Male		19	14	5	5	p=0.03
Female		24	12	10	23	x²=8.3

	Patients	Controls	Statistics	Abused	Non- Abused	Statistics
Male	10	33	p=0.009	20	23	p=0.5
Female	33	36	x²=6.7	36	33	x²=0.3

3.2.10 Age

Age of subjects is shown in Table 3.13. There was no difference among the 4 groups in terms of age ($p=0.9$, $df=3$, Mean ranks / CB =42.9, CA=45.2, PB= 54.0, PA=55.2).

There was a significant difference between controls and MDD patients in terms of age, we had older patients compared to the controls ($p=0.02$, $z=-1.6$, Mean Rank / Controls=43.7, Patients=54.6).

There was no difference between abused and non-abused group in terms of age ($p=0.6$, $z=-0.4$, Mean Rank/ Abused=47.9, Non-Abused=45.3). Table 3.13

3.2.10.1 Correlation between age and CAR

The correlation between age of the subjects and cortisol at individual time points was positive and significant at time of awakening. When looking at AUC, the correlation was not significant (cortisol0- $p=0.01$, $r=0.2$) Table 3.12

Table 3.12 Correlation between cortisol at all individual time points, AUC, and age

	cortisol0	cortisol 15	cortisol 30	cortisol 45	cortisol 60	cortisol 90
Age (years)	p=0.01	p=0.1	p=0.3	p=0.1	p= 0.1	p=0.5
	r=0.2	r=0.1	r=0.09	r=0.1	r=0.1	r=0.05
	AUCg60		AUCi60		AUCg90	
	p=0.1		p=0.6		p=0.2	
	r=0.1		r=-0.04		r=0.1	
					p=0.6	
					r=-0.04	

3.2.11 Body Mass Index (BMI)

The BMI of subjects is shown in Table 3.13. There was no difference among the 4 groups in terms of BMI ($p=0.3$, $df= 3$, Mean Ranks/ CB=44.08, CA=43.30, PB=50.7, PA=57.6).

There was no difference between MDD patients and healthy controls in terms of BMI ($p=0.09$, $z=-1.6$, Mean Ranks/ Controls=43.7, Patient=54.6).

There was no difference between abused and non-abused groups in terms of BMI ($p=0.6$, $z=-0.46$, Mean Ranks / Abused=47.9, Non-abused=45.3) Table 3.13

Table 3.13 Age and BMI

			CB	CA	PB	PA	Stat		
AGE		Median,(IQR)	48,(22)	47.5,(19)	55,(24)	50,(20)	P=0.9		
		Mean±SD	43.8± 13.7	46.3±11.4	52.3±13.3	50.1± 12.4			
BMI (Kg/m²)		Median,(IQR)	25.1,(6)	24.3,(8)	30,(11)	27.7,(5)	P=0.3		
		Mean±SD	25.6 ± 4.4	26.2 ± 5.1	27.4±5.7	27.6± 4.1			
		Patients		Controls	Stat	Abused	Non-Abused	Stat	
AGE	Median,(IQR)		52,(21)		48,(20)	P=0.02	49,(23)	50,(17)	P=0.6
	Mean±SD		50.9±12.6		44.7± 2.8		48.0±12.2	46.1±13.9	
BMI (Kg/m²)		Median,(IQR)	28.5,(6)		25,(5)	P=0.09	26.1,(7)	26.2,(8)	P=0.6
		Mean±SD	27.5± 4.8		25.8±4.7		26.6±4.9	26±4.6	

3.2.11.1 Correlation between BMI and CAR

The correlation between BMI and CAR was not significant at any time points

Table 3.14

Table 3.14 Correlation between cortisol at all individual time points, AUC, and BMI

	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
BMI	p=0.9 r=0.1	p=0.5 r=-0.09	p=0.3 r=-0.05	p=0.3 r=-0.09	p=0.1 r=-0.1	p=0.2 r=-0.1
	AUCg60		AUCi60		AUCg90	
BMI	p=0.4 r=-0.08		p=0.1 r=-0.1		p=0.2 r=-0.1	

3.2.12 CAR

Cortisol30 was appeared as the highest cortisol among all of the time points (nmol/L) .Table 3.15

Table 3.15 Cortisol-All the time points

	Median, (IQR)	Mean±SD
cortisol0	10.3, (8.2)	11.3±6.2
cortisol15	13.9, (9.9)	13.5±6.9
cortisol30	15.10, (11.9)	15.3±8.2
cortisol45	12.30, (11.7)	13.9±7.5
cortisol60	11.80, (9.5)	12.2±7.2
cortisol90	9.3, (8.5)	10.7±6.6

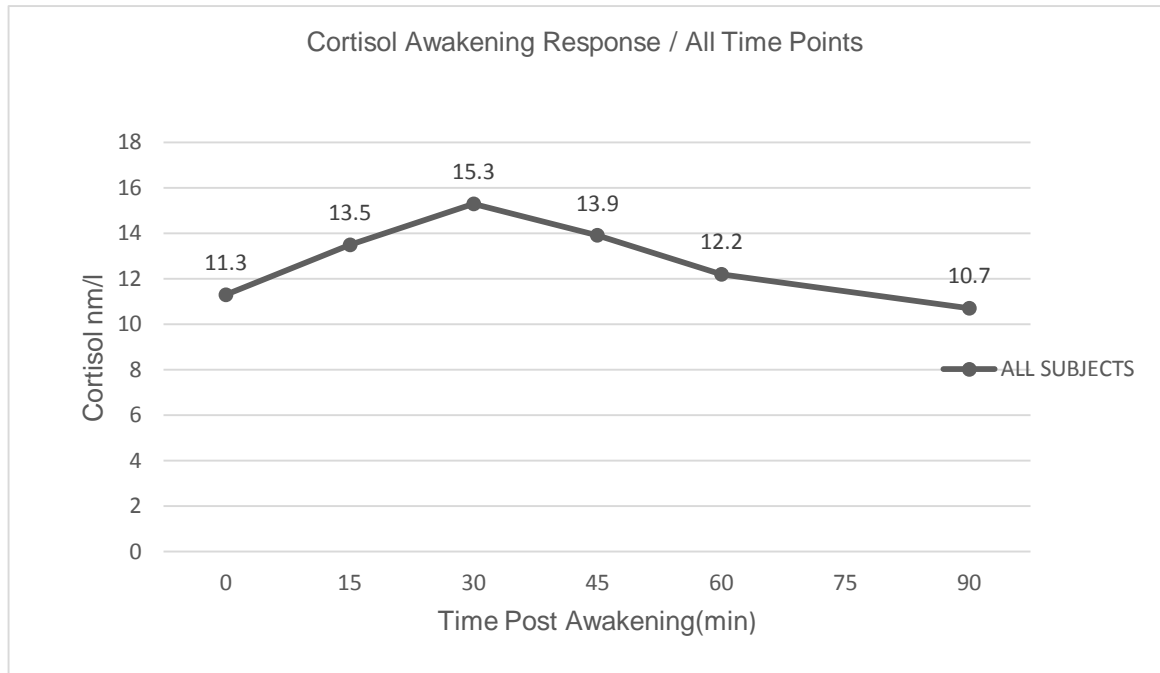


Figure 3-1 Cortisol-All time points

3.2.12.1 Comparing cortisol at all individual time points between MDD patients and healthy controls

Cortisol at all individual time points was higher in MDD patients compared with controls and the changes was statistically significant at 15, 30, 45 and 60 minutes after awakening (cortisol15- $p=0.01$, $z=-2.3$, Mean Rank=65.6; cortisol30- $p=0.03$, $z=-2.1$, Mean Rank=64.6; cortisol45- $p=0.009$, $z=-2.6$, Mean Rank=66.70; cortisol60- $p=0.02$, $z=-2.2$, Mean Rank=65.3).

There was no difference in cortisol at 0 and at 90 minutes between MDD patients and healthy controls (cortisol0- $p=0.3$, $z=-0.9$; cortisol90- $p=0.1$, $z=-1.6$) Table 3.16

Table 3.16 Comparing cortisol at all individual time points between MDD patients and healthy controls

	Patients		Controls		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
cortisol0	11.1,(8)	11.8±5.9	9.4,(8)	11.0±6.5	0.3
cortisol15	15.6,(8)	15.0±6.0	11.7,(10)	12.6±7.2	0.01
cortisol30	17.3,(10)	16.6±7	12.9,(11)	14.4±8.8	0.03
cortisol45	16.5,(12)	16.1±7.1	10.4,(7)	12.5±7.4	0.009
cortisol60	13.7,(10)	14.0±7.4	10.0,(9)	11.0±6.8	0.02
cortisol90	11.0,(9)	11.9±7.1	8.7,(8)	9.9±6.2	0.1

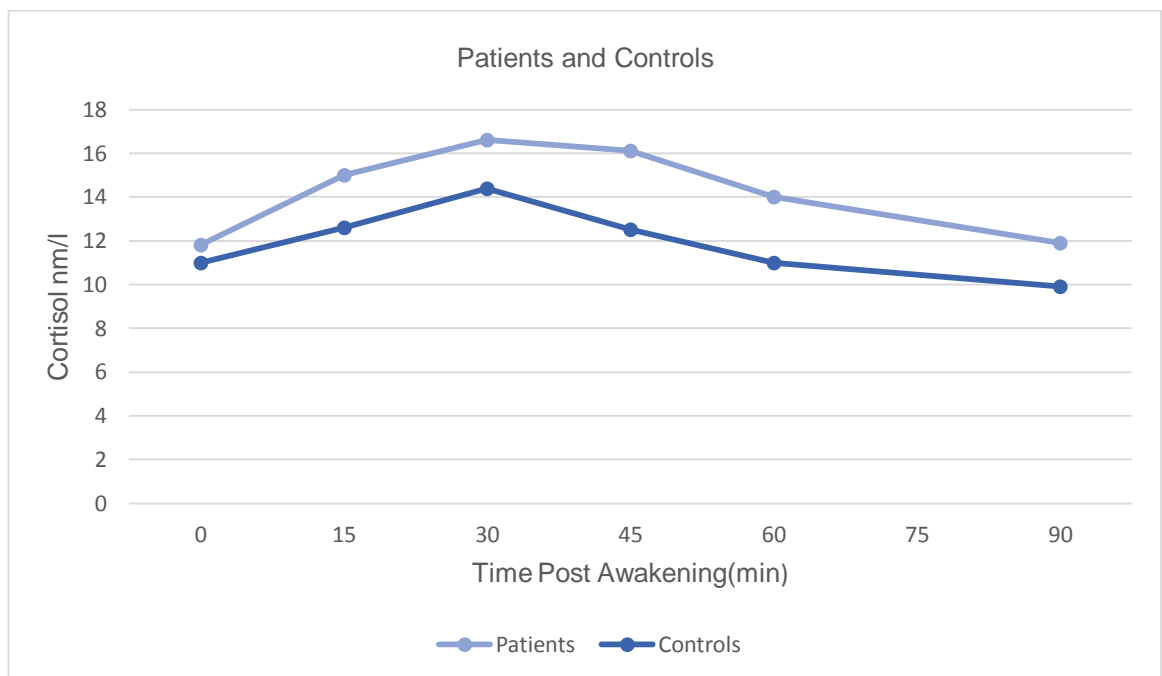


Figure 3-2 Cortisol-MDD patients and healthy controls

3.2.12.2 Comparing cortisol at all individual time points between abused and non-abused subjects

There was no difference between abused and non-abused groups (cortisol0- $p=0.8$, $z=-0.1$, Mean Rank=56.9; cortisol15- $p=0.2$, $z=-1.0$, Mean Rank=59.8, cortisol30- $p=0.09$, $z=-1.6$, Mean Rank=61.6; cortisol45- $p=0.6$, $z=-1.8$, Mean

Rank=62.2; cortisol60-p=0.1, z=-1.3, Mean Rank=60.6; cortisol90-p=0.4, z=-0.8, Mean Rank=59.0) Table 3.17

Table 3.17 Comparing cortisol at all individual time points between abused and non-abused subjects

	Abused		Non-Abused		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
cortisol0	10.35,(9)	11.7±6.9	10.4,(7)	11.0±5.6	0.8
cortisol15	14.6,(13)	14.2±7.1	11.9,(9)	12.9±6.6	0.2
cortisol30	16.8,(12)	16.4±8.2	12.9,(9)	14.1±8.1	0.09
cortisol45	15.2,(11)	14.9±7.1	10.8,(8)	12.8±7.8	0.6
cortisol60	13.3,(10)	13.0±7.5	10.8,(8)	11.3±6.8	0.1
cortisol90	9.8,(8)	11.1±6.8	8.8,(8)	10.2±6.4	0.4

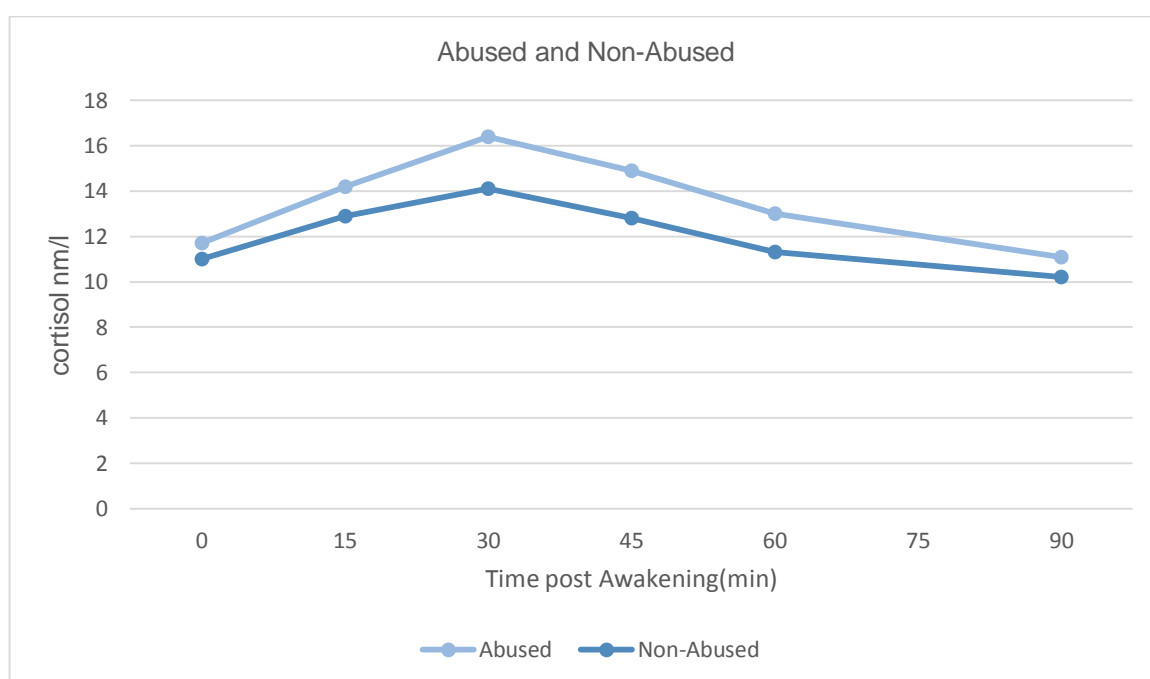


Figure 3-3 Cortisol-Abused and non-Abused

3.2.12.3 Comparing cortisol at all individual time points between healthy controls with a history of childhood trauma and healthy controls without a history of childhood trauma (CA compared with CB)

There was no difference in cortisol between the two groups (CA compared with CB).

(cortisol0-p=0.8, z=-0.2, Mean Rank CB=34.2, cortisol15-p=0.6, z=-0.2, Mean Rank=33.5; cortisol-p=0.8, z=-0.2, Mean Rank=35.6; cortisol45-p=0.6, z=-0.6, Mean Rank=36.2; cortisol60-p=0.7, z=-0.4, Mean Rank=35.8; cortisol90-p=0.8, z=-0.2, Mean Rank=35.7).Table 3.18

Table 3.18 Comparing cortisol at all individual time points between healthy controls with a history of childhood trauma and healthy controls without a history of childhood trauma (CA compared with CB)

	CB		CA		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
cortisol0	9.7,(7)	11.0± 5.9	9.3,(9)	11.1±7.5	0.8
cortisol15	11.4,(9)	12.7±7.0	12.9,(9)	12.5±7.8	0.6
cortisol30	12.9,(8)	14.1±8.5	12.6,(13)	14.9±9.4	0.8
cortisol45	10.3,(6)	12.4±7.9	10.4,(10)	12.6±6.5	0.6
cortisol60	9.8,(6)	11.0±6.09	11.1,(11)	11.1± 6.8	0.7
cortisol90	8.2,(7)	9.7±6.0	9.1,(9)	10.2±6.5	0.8

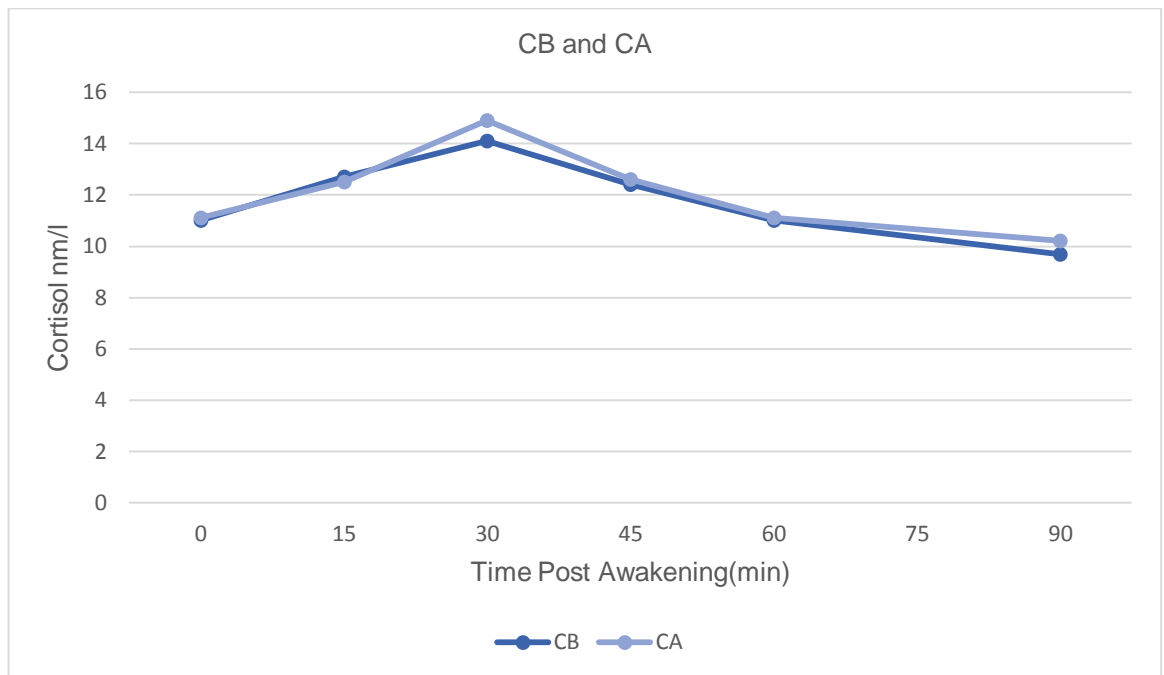


Figure 3-4 Cortisol-Healthy controls with a history of childhood trauma and healthy controls without a history of childhood trauma (CA compared with CB)

3.2.12.4 Comparing cortisol at all individual time points between MDD patients with a history of childhood trauma and MDD patients without a history of childhood trauma (PA compared with PB)

There were significant changes in cortisol at the time points of 15 minutes (cortisol15- $p=0.04$, $z=-2$, Mean Rank=24.8), and 45 minutes (cortisol45- $p=0.03$, $z=-2.1$, Mean Rank=24.9). Table 3.19

Although cortisol appears to be higher in the PA group, there was no significant difference at awakening time (cortisol0- $p=0.2$, $z=-1.0$, Mean Rank=23.4), 30 minutes (cortisol- $p=0.05$, $z=-1.9$, Mean Rank=24.7), 60 minutes (cortisol60- $p=0.1$, $z=-1.4$, Mean Rank=24.0) and 90 minutes after awakening (cortisol 90- $p=0.3$, $z=-0.9$, Mean Rank=23.3).Table 3.19

Table 3.19 Comparing cortisol at all individual time points between MDD patients with a history of childhood trauma and MDD patients without a history of childhood trauma (PA compared with PB)

	PB		PA		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
cortisol0	11,(6)	10.2±4.1	11,(10)	12.7 ± 6.6	P=0.2
cortisol15	14.2,(8)	12.7±6.0	16.9,(6)	16.3±5.8	P=0.04
cortisol30	13,(8)	13.9±6.4	19,(8)	18.1±7.07	P=0.05
cortisol45	12.5,(10)	13.3±5.9	19.05,(12)	17.6±7.4	P=0.03
cortisol60	12.5,(11)	11.8±5.9	14.7,(10)	15.2±8.02	P=0.1
cortisol90	9.9,(11)	10.2±6.1	11.3,(10)	12.8±7.6	P=0.3

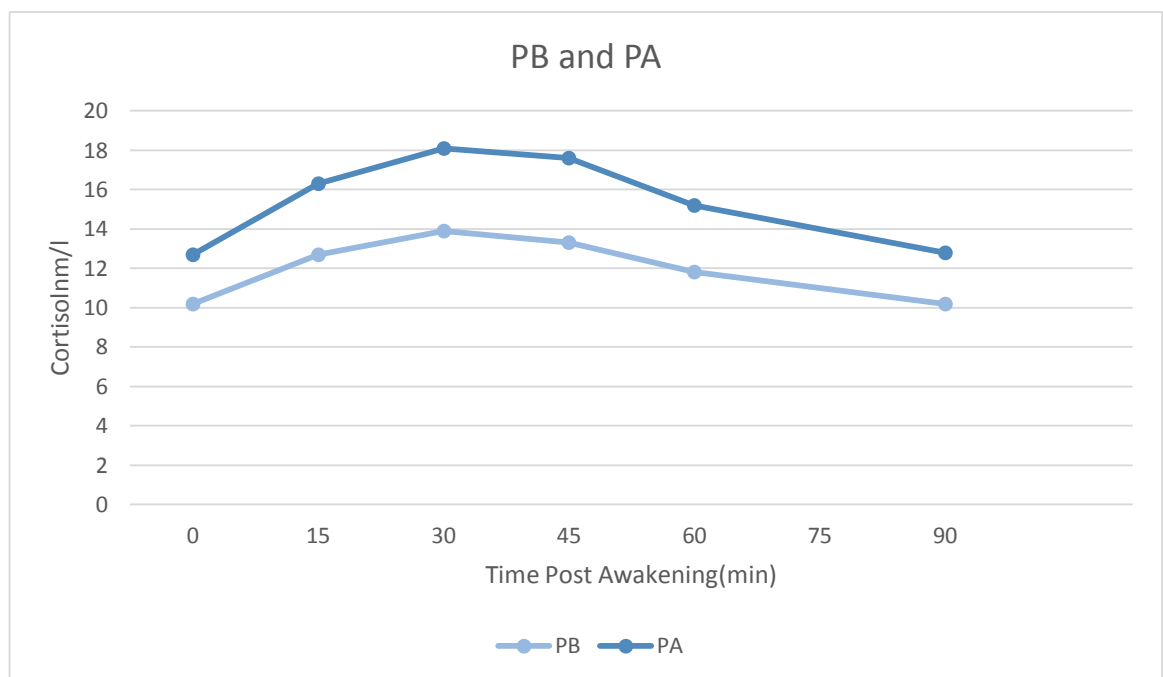


Figure 3-5 Cortisol-MDD patients with a history of childhood trauma and MDD patients without a history of childhood trauma (PA compared with PB)

3.2.12.5 Comparing cortisol at all individual time points between healthy controls with a history of childhood trauma and MDD patients with a history of childhood trauma (CA compared with PA)

Cortisol was higher in the PA group compared with the CA group and the changes were significant at cortisol15 (cortisol15-p=0.01, z=-2.4, Mean Rank=32.6) and at cortisol45 (cortisol 45-p=0.02, z= -2.3, Mean Rank=32.2).

At time of awakening (cortisol0-p=0.2, z=-1.0, Mean Rank=29.6), at 30 (cortisol30-p=0.06, z=-1.8, Mean Rank=31.2), at 60 (cortisol60-p=0.07, z=-1.7, Mean Rank=31.1) and at 90 minutes after awakening (cortisol 90-p=0.1, z=-1.3, Mean Rank=30.3) there was no difference Table 3.20

Table 3.20 Comparing cortisol at all individual time points between healthy controls with a history of childhood trauma and MDD patients with a history of childhood trauma (CA compared with PA)

	CA		PA		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
cortisol0	9.3,(9)	11.1±7.5	11,(10)	12.7 ± 6.6	0.2
cortisol15	12.9,(9)	12.5±7.8	16.9,(6)	16.3±5.8	0.01
cortisol30	12.6,(13)	14.9±9.4	19,(8)	18.1±7.07	0.06
cortisol45	10.4,(10)	12.6±6.5	19.05,(12)	17.6±7.4	0.02
cortisol60	11.1,(11)	11.1± 6.8	14.7,(10)	15.2±8.02	0.07
cortisol90	9.1,(9)	10.2±6.5	11.3,(10)	12.8±7.6	0.1

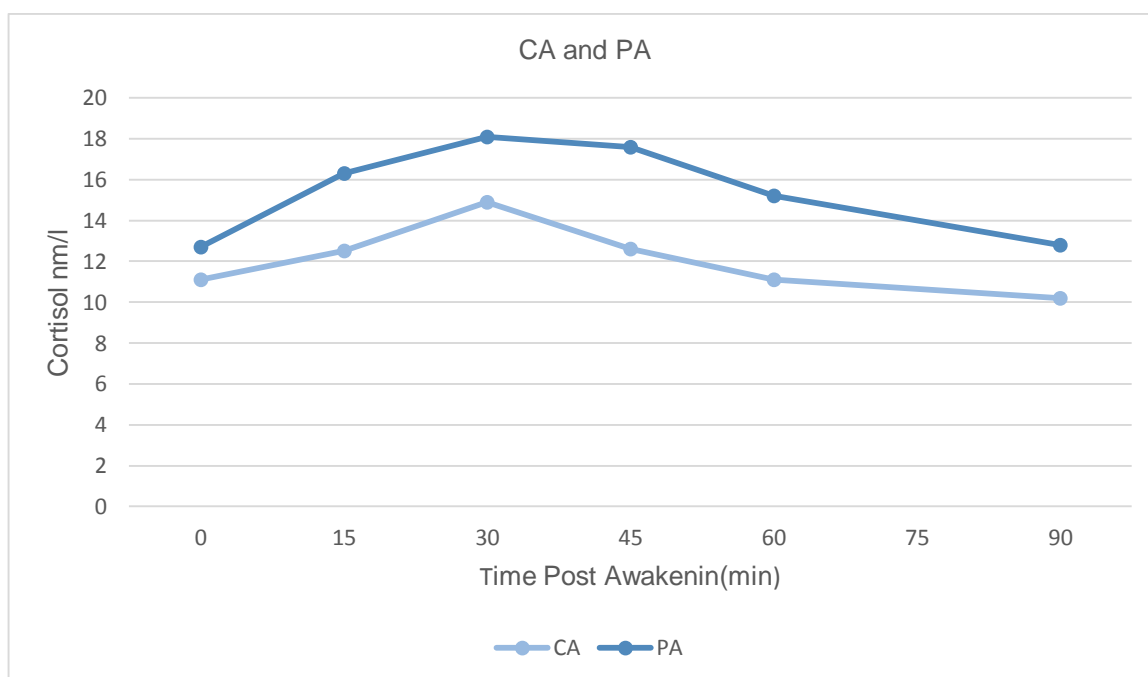


Figure 3-6 Cortisol-Healthy controls with a history of childhood trauma and MDD patients with a history of childhood trauma (CA compared with PA)

3.2.12.6 Comparing cortisol at all individual time points between healthy controls without a history of childhood trauma and MDD patients without a history of childhood trauma (CB compared with PB)

There were no statistically significant differences at any time point in the PB group when compared with the CB group.

(cortisol0-p=0.9, z=-0.07, Mean Rank=29.7; cortisol15-p=0.7, z=-0.3, Mean Rank=30.7; cortisol30-p=0.7, z=-0.3, Mean Rank=30.8; cortisol45-p=0.4, z=-0.9, Mean Rank=32.3; cortisol60-p=0.3, z=-0.3, Mean Rank=33; cortisol90-p=0.7, z=-0.6, Mean Rank=30.8).Table 3.21

Table 3.21 Comparing cortisol at all individual time points between healthy controls without a history of childhood trauma and MDD patients without a history of childhood trauma (CB compared with PB)

	CB		PB		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
cortisol0	9.7,(7)	11.0± 5.9	11,(6)	10.2±4.1	0.9
cortisol15	11.4,(9)	12.7±7.0	14.2,(8)	12.7±6.0	0.7
cortisol30	12.9,(8)	14.1±8.5	13,(8)	13.9±6.4	0.7
cortisol45	10.3,(6)	12.4±7.9	12.5,(10)	13.3±5.9	0.4
cortisol60	9.8,(6)	11.0±6.09	12.5,(11)	11.8±5.9	0.3
cortisol90	8.2,(7)	9.7±6.0	9.9,(11)	10.2±6.1	0.7

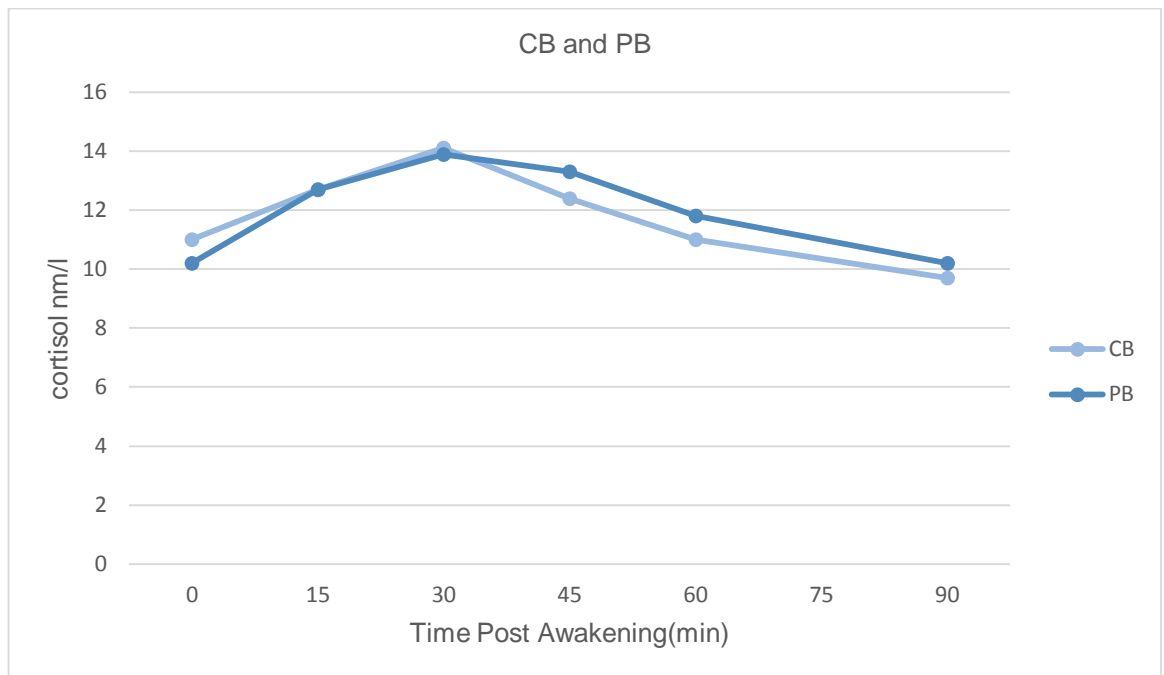


Figure 3-7 Cortisol-Healthy controls without a history of childhood trauma and MDD patients without a history of childhood trauma (CB compared with PB)

3.2.12.7 Comparing cortisol at all individual time points between MDD patients with a history of childhood trauma and healthy controls without a history of childhood trauma (PA compared with CB)

Cortisol appears higher in the PA group when compared with the CB group, and the changes were significant at all-time points except at time of awakening and at 90 min after awakening.

(cortisol0- $p=0.2$, $z=-1.0$, Mean Rank=39.2; cortisol15- $p=0.01$, $z=-2.4$, Mean Rank=43.4; cortisol30- $p=0.008$, $z=-2.6$, Mean Rank=44.0; cortisol45- $p=0.003$, $z=-2.9$, Mean Rank=44.9; cortisol60- $p=0.01$, and $z=-2.5$, Mean Rank=43.5; cortisol90- $p=0.06$, $z=-1.8$, Mean Rank=41.6).Table 3.22

Table 3.22 Comparing cortisol at all individual time points between MDD patients with a history of childhood trauma and healthy controls without a history of childhood trauma (PA compared with CB)

	CB		PA		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
cortisol0	9.7,(7)	11.0±5.9	11,(10)	12.7 ± 6.6	0.2
cortisol15	11.4,(9)	12.7±7.0	16.9,(6)	16.3±5.8	0.01
cortisol30	12.9,(8)	14.1±8.5	19,(8)	18.1±7.07	0.008
cortisol45	10.3,(6)	12.4±7.9	19.05,(12)	17.6±7.4	0.003
cortisol60	9.8,(6)	11.0±6.09	14.7,(10)	15.2±8.02	0.01
cortisol90	8.2,(7)	9.7±6.0	11.3,(10)	12.8±7.6	0.06

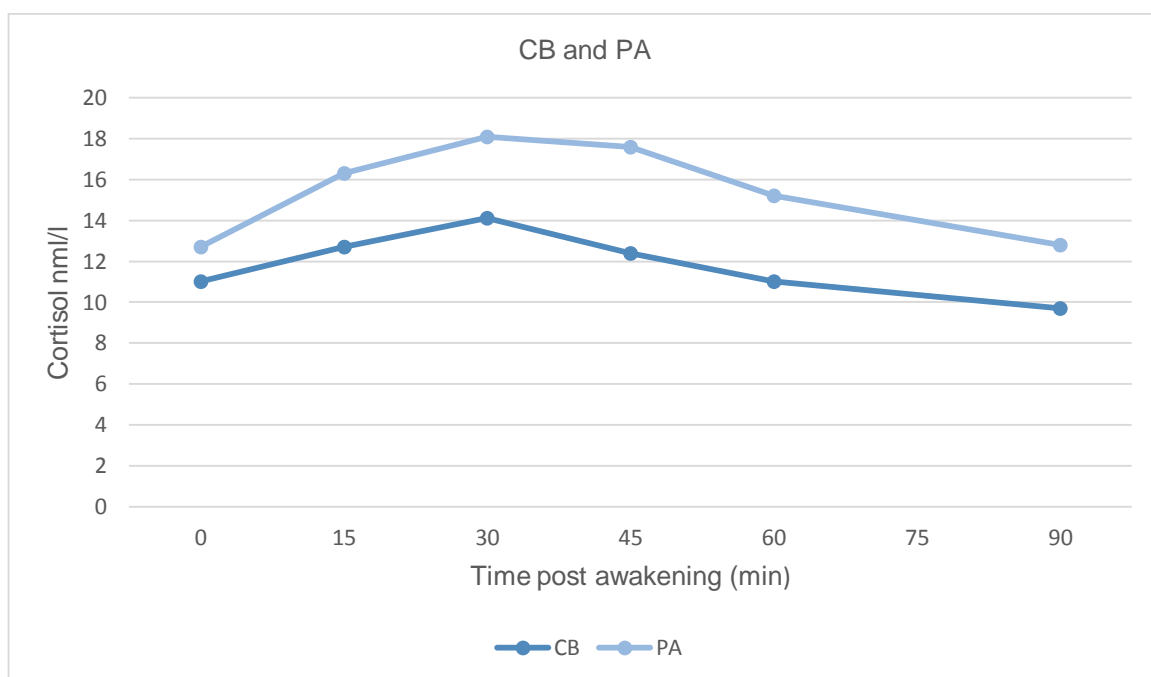


Figure 3-8 Cortisol-MDD patients with a history of childhood trauma and healthy controls without a history of childhood trauma (PA compared with CB)

3.2.12.8 Comparing cortisol among all the groups (CB, CA, PA, and PB)

There were significant changes in cortisol among the four groups (Kruskal-Wallis test) at 15 and 45 minutes post awakening but not at awakening time, 30, 60 and 90 minutes after awakening (cortisol-p=0.6; cortisol15-p=0.03; cortisol30-p=0.05; cortisol45-p=0.01; cortisol60-p=0.07, cortisol90-p=0.3, df= 3).

The PA group had the highest cortisol at each time point compared with the other groups. PA (Mean Rank)/ cortisol0 (63.3), cortisol15 (71.8), cortisol 30(71.0), cortisol45 (73.2), cortisol60 (69.7), cortisol90 (66.3). Table 3.23

Table 3.23 Comparing cortisol among all the groups

	CB		CA		PB		PA		P
cortisol 0	9.7	11.0± 5.9	9.3	11.1±7.5	11	10.2±4.1	11	12.7 ± 6.6	0.6
cortisol15	11.4	12.7±7.0	12.9	12.5±7.8	14.2	12.7±6.0	16.9	16.3±5.8	0.03
cortisol30	12.9	14.1±8.5	12.6	14.9±9.4	13	13.9±6.4	19	18.1±7.07	0.05
cortisol45	10.3	12.4±7.9	10.4	12.6±6.5	12.5	13.3±5.9	19.05	17.6±7.4	0.01
cortisol60	9.8	11.0±6.09	11.1	11.1± 6.8	12.5	11.8±5.9	14.7	5.2±8.02	0.07
cortisol90	8.2	9.7±6.0	9.1	10.2±6.5	9.9	10.2±6.1	11.3	12.8±7.6	0.3

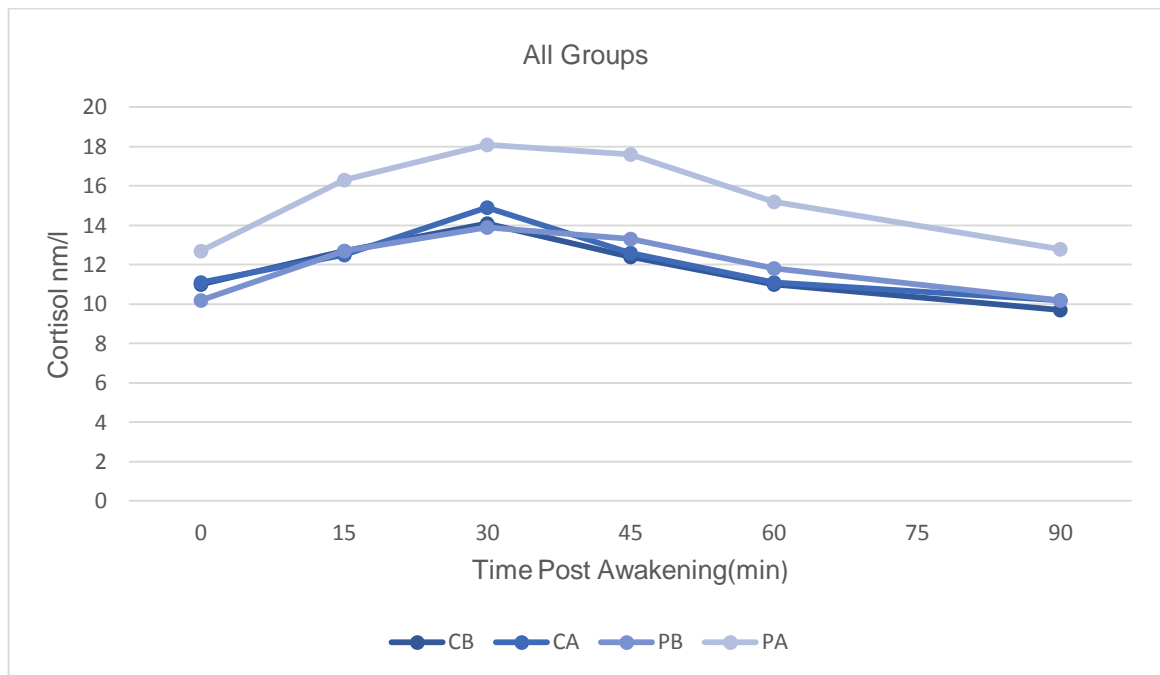


Figure 3-9 Cortisol-All the groups

3.2.13 Area Under the Curve (AUC) CAR

AUC_g and AUC_i-CAR- for all subjects are presented below Table 3.24

Table 3.24 AUC_g and AUC_i

	Mean	Median,(IQR)
AUC _g 60	819.3±392.3	836.2,(607)
AUC _i 60	135.3±312.4	100.1,(338)
AUC _g 90	1161.7±568.3	1145.6,(842)
AUC _i 90	351.8±547.7	258.0,(680)

3.2.13.1 Comparing AUC_i and AUC_g between MDD patients and healthy controls

We looked at both of the AUC measures in two ways. First, AUC values were calculated using all time points up to 60 minutes after awakening (AUC₆₀), in order to obtain results compatible with the method used in most previous studies, which have generally measured only the first 60 minutes after awakening. Second, we calculated the AUC using in addition the cortisol measure at 90

minutes after awakening (AUC90), to give the fullest account of the overall dynamic of the cortisol.

There was a statistically significant difference between MDD patients and controls when taken together, (AUCg60-p=0.01; AUCg-90-p=0.02; AUCg60-z=-2.4; and AUCg90-z=-2; AUCi60-p=0.03; AUCi60-z=-2.1). Patients had higher AUCg60, 90 (Mean Rank= 66.1, 65.2) and AUCi60 (Mean Rank=64.8).Table 3.25

Table 3.25 Comparing AUCg and AUCi between MDD patients and healthy controls

	Patients		Controls		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
AUCg60	982.5,(483)	913±358.5	677.2,(511)	760.9±403.6	0.01
AUCi60	205.5,(426)	199.2±315.7	68.2,(304)	95.5±305.9	0.03
AUCg90	1374,(789)	1298.8±554.3	888,(727)	1076.2±564.0	0.02
AUCi90	481.5,(704)	464.3±561.8	217.5,(623)	281.7 ± 530.9	0.05

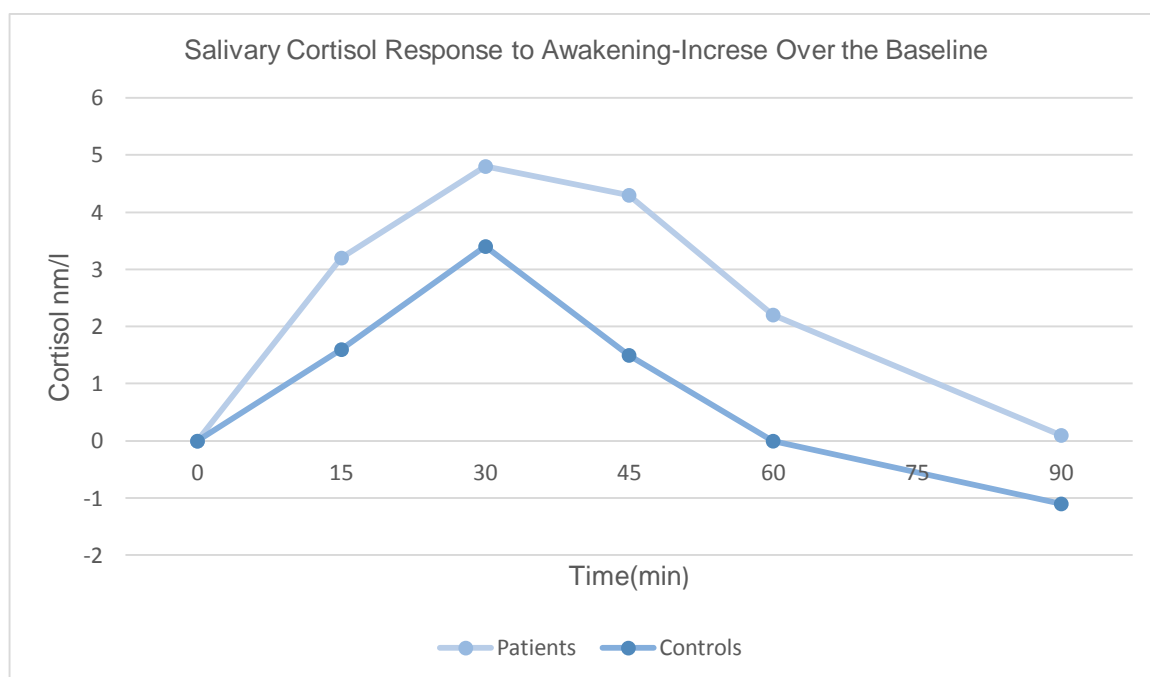


Figure 3-10 Salivary cortisol response to awakening-increase over the baseline in healthy controls and MDD patients

3.2.13.2 Comparing AUCi and AUCg between abused and non-abused subjects

The AUCg and AUCi were not significantly higher in abused individuals compared with non-abused individuals when patient and control groups were combined (AUCg60-p=0.1; AUCg90-p=0.1; AUCg-60-z=-1.5 ; AUCg90-z=1.5; Mean Rank AUCg60=61.4; and Mean Rank AUCg90=61.3; AUCi60-p=0.2; AUCi90-p=0.3; AUCi60-z=-1.2; AUCi90-z=1.0; Mean Rank AUCi60= 60.2; Mean Rank AUCi90= 61.3) (Table 3.26).

Table 3.26 Comparing AUCg and AUCi between abused and non-abused subjects

	Abused		Non-Abused		p
	Median,(IQR)	Mean±SD	Median(IQR)	Mean±SD	
AUCg60	962.2,(656)	870.9±389.1	702.3,(510)	767.6±392.2	0.1
AUCi60	178.5,(360)	168.3±324.3	70.8,(295)	102.4±299.3	0.2
AUCg90	1314.7,(893)	1234.3±573.6	10.6,(721)	1089.1±558.6	0.1
AUCi90	404.2,(731)	410.7±565.4	212.6,(615)	292.9±527.9	0.3

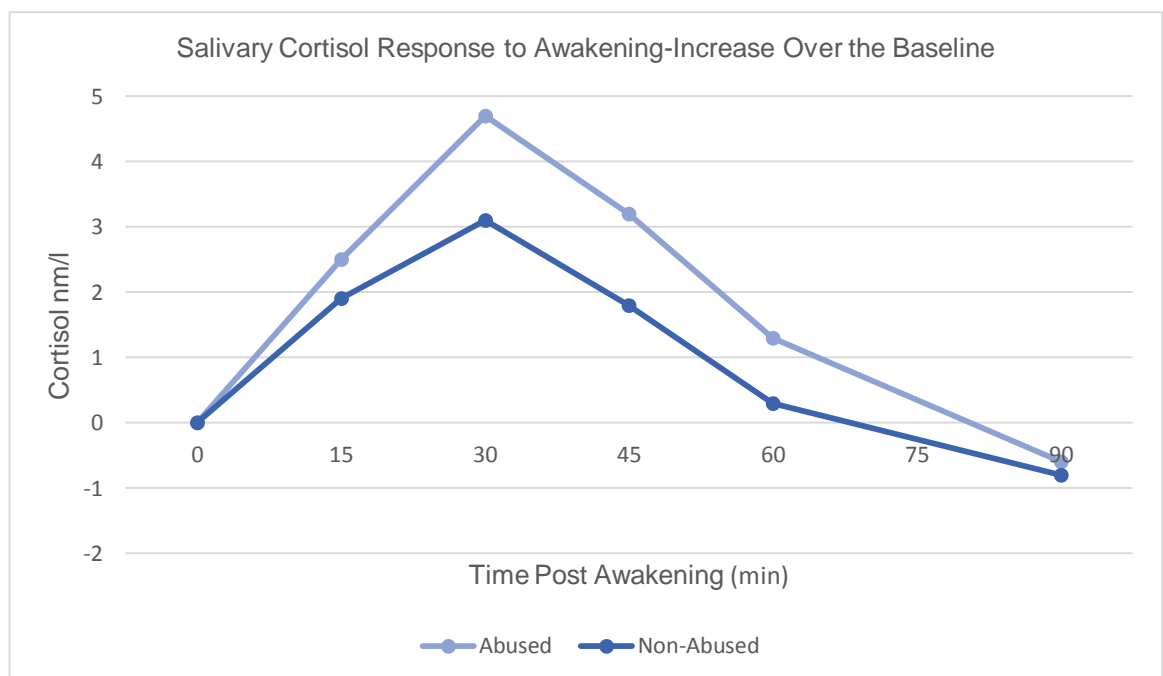


Figure 3-11 Salivary cortisol response to awakening-Increase over the baseline in abused and non-abused

3.2.13.3 Comparing AUCi and AUCg between healthy controls without a history of childhood trauma and healthy controls with a history of childhood trauma (CB compared with CA)

There was no difference--AUCg60-p=0.9; AUCg90-p=0.8; AUCg60-z=-0.006; AUCg90-z=-0.2; and AUCi60-p=0.7; AUCi90-p=0.7; AUCi60-z=-0.2; AUCg60-z=0.2) --between abused controls group and non-abused controls group. The mean of AUC was marginally higher in the CA group compared with the CB group but the changes were not significant (Mean Rank AUCg60= 35.0; Mean Rank AUCg90=35.7; Mean Rank AUCi60=34.0; Mean Rank AUCi90=34.1) Table 3.27

Table 3.27 Comparing AUCg and AUCi between healthy controls without a history of childhood trauma and healthy controls with a history of childhood trauma (CB compared with CA)

	CB		CA		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
AUCg60	606.7,(511)	750±408.85	721.8,(556)	768.9±402.7	0.9
AUCi60	81.0,(216)	93.5±307	34.1,(397)	98.8±310.1	0.7
AUCg90	888.0,(697)	1068.1±569.7	993.,7(840)	1089.6±565.4	0.8
AUCi90	217.5,(421)	276.4±537.8	197.6,(707)	290.4±529.6	0.7

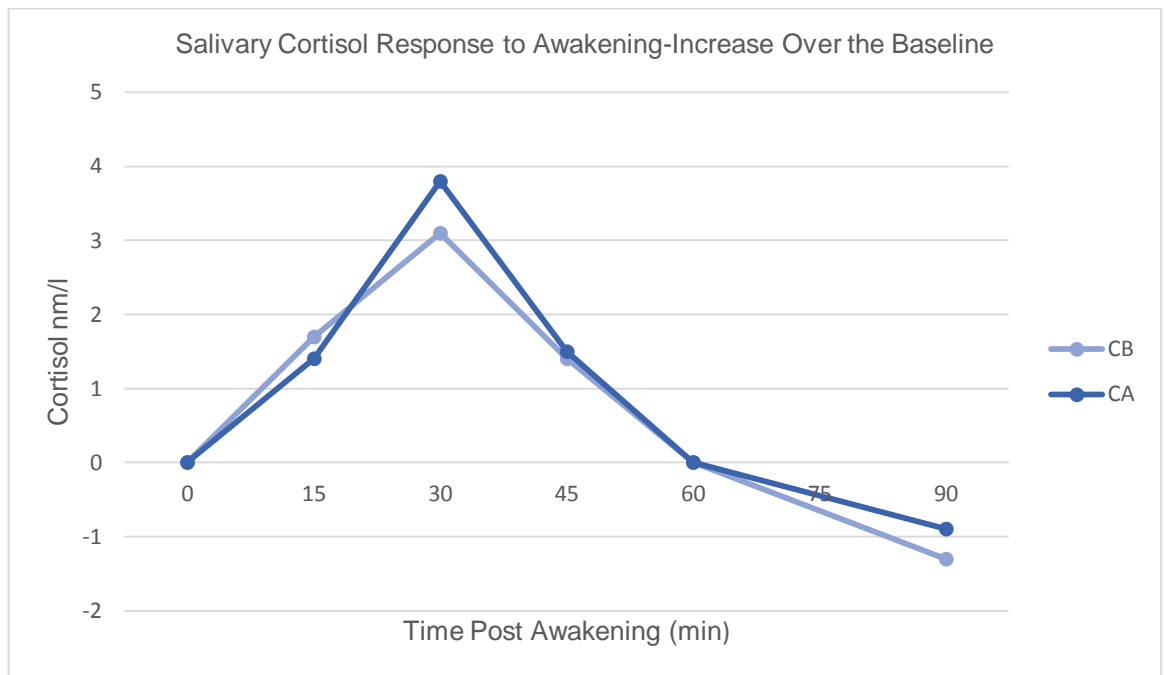


Figure 3-12 Salivary cortisol response to awakening-Increase over the base line in healthy controls without a history of childhood trauma and healthy controls with a history of childhood trauma (CB compared with CA)

3.2.13.4 Comparing AUCg and AUCi between MDD patients without a history of childhood trauma and MDD patients with a history of childhood trauma (PB compared with PA)

The AUCg60 was significantly higher in the PA group compared with the PB group ($p=0.04$, $z=-2.0$), (Mean Rank: AUCg60=24.8) (Table 3.28)

Table 3.28 Comparing MDD patients without a history of childhood trauma and MDD patients with a history of childhood trauma (PB compared with PA)

	PB		PA		p
	Median,(IQR)	Mean \pm SD	Median,(IQR)	Mean \pm SD	
AUCg60	819.7,(625)	766.6 \pm 314.2	1072,(398)	991.4 \pm 361.2	0.04
AUCi60	74.2,(432)	151.4 \pm 228.7	220.1,(411)	224.9 \pm 354.9	0.2
AUCg90	1112.2,(962)	1086.5 \pm 469.5	1431,(743)	1412.6 \pm 570.3	0.06
AUCi90	171,(688)	358.3 \pm 417.6	519,(708)	521.1 \pm 625.2	0.2

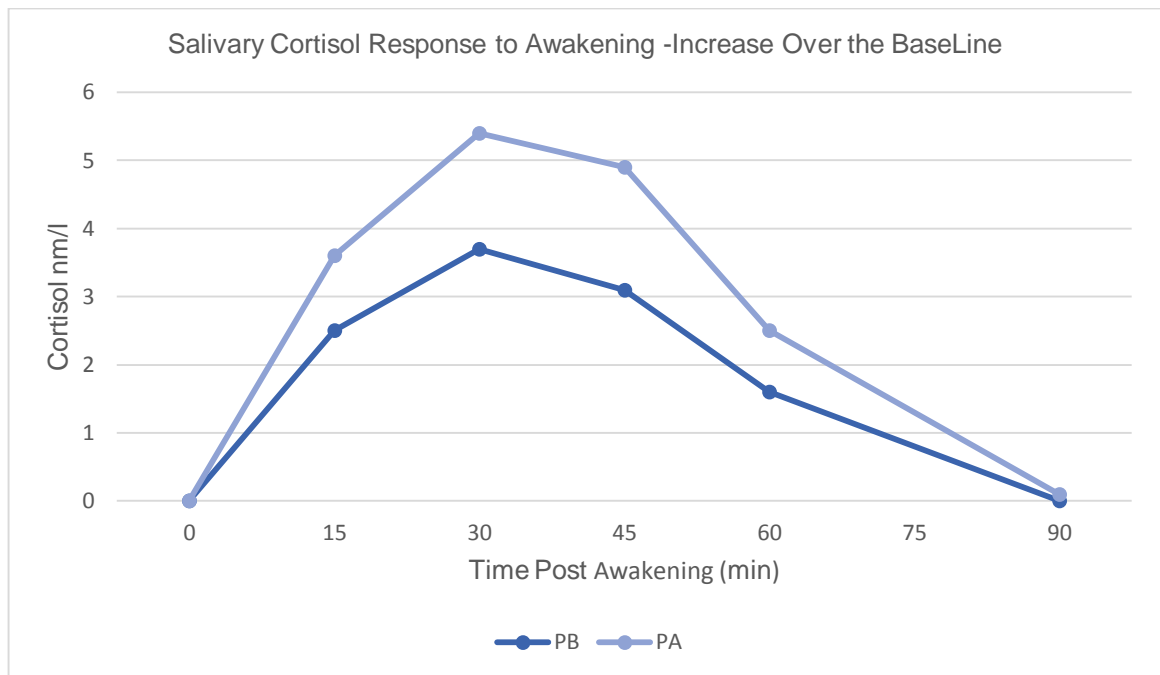


Figure 3-13 Salivary cortisol response to awakening-Increase over the baseline in MDD patients without a history of childhood trauma and MDD patients with a history of childhood trauma (PB compared with PA)

3.2.13.5 Comparing AUCg and AUCi between healthy controls with a history of childhood trauma and MDD patients with a history of childhood trauma (CA compared with PA)

There was a significant difference between healthy controls with history of childhood trauma and MDD patients with a history of childhood trauma (AUCg60- $p=0.02$; and AUCg90- $p=0.03$; AUCg60- $z=-2.3$; AUCg90- $z=2.0$; AUCi60- $p=0.05$, AUCi90- $p=0.07$; AUCi60- $z=-1.9$; AUCi90- $z=-1.7$), with the MDD patient groups showing higher AUCg60 and AUCg90 (Mean Rank AUCg60=32.2; Mean Rank AUCg90= 31.7) Table 3.29

Table 3.29 Comparing AUCg and AUCi between healthy controls with a history of childhood trauma and MDD patients with a history of childhood trauma (CA compared with PA)

	CA		PA		p
	Median,(IQR)	Mean \pm SD	Median,(IQR)	Mean \pm SD	
AUCg60	721.8,(556)	768.9 \pm 402.7	1072,(398)	991.4 \pm 361.2	0.02
AUCi60	34.1,(397)	98.8 \pm 310.1	220.1,(411)	224.9 \pm 354.9	0.05
AUCg90	993.7,(840)	1089.6 \pm 565.4	1431,(743)	1412.6 \pm 570.3	0.03
AUCi90	197.6,(767)	290.4 \pm 529.6	519,(708)	521.1 \pm 625.2	0.07

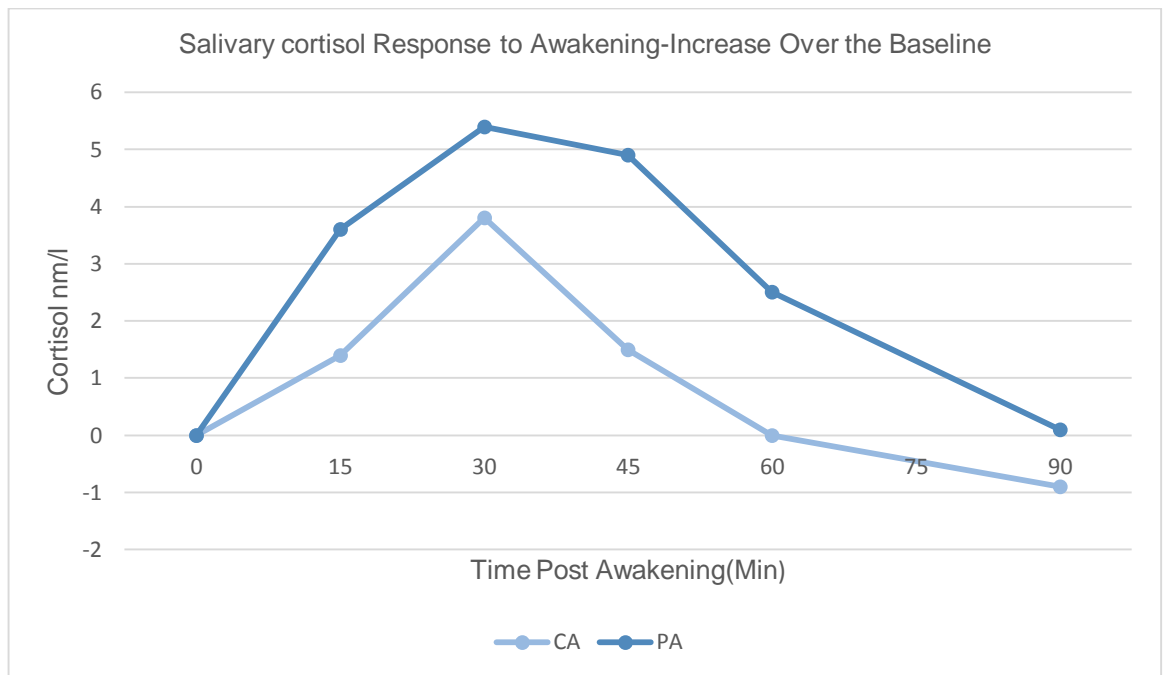


Figure 3-14 Salivary cortisol response to awakening-Increase over the baseline in healthy controls with a history of childhood trauma and MDD patients with a history of childhood trauma (CA compared with PA)

3.2.13.6 Comparing AUCg and AUCi between healthy controls without a history of childhood trauma and MDD patients without a history of childhood trauma (CB compared with PB)

There was no significant difference between these two groups although both AUCg and AUCi appeared numerically higher in the patient group (AUCg60- $p=0.6$; and AUCg90- $p=0.6$; AUCg60- $z=-0.4$; and AUCg90- $z=-0.4$; Mean Rank AUCg60=31.3; and Mean Rank AUCg90=31.0; AUCi60- $p=0.6$; AUCi90- $p=0.7$; AUCi60- $z=-0.4$; AUCi90- $z=-0.2$; Mean Rank AUCi60=31.2; Mean Rank AUCi90=30.6) Table 3.30

Table 3.30 Comparing AUCg and AUCi between healthy controls without a history of childhood trauma and MDD patients without a history of childhood trauma (CB compared with PB)

	CB		PB		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
AUCg60	606.7,(511)	750±408.85	819.7,(625)	766.6±314.2	0.6
AUCi60	81.0,(216)	93.5±307	74.2,(432)	151.4±228.7	0.6
AUCg90	888.0,(697)	1068.1±569.7	1112.2,(962)	1086.5±469.5	0.6
AUCi90	217.5,(421)	276.4±537.8	171,(688)	358.3±417.6	0.7

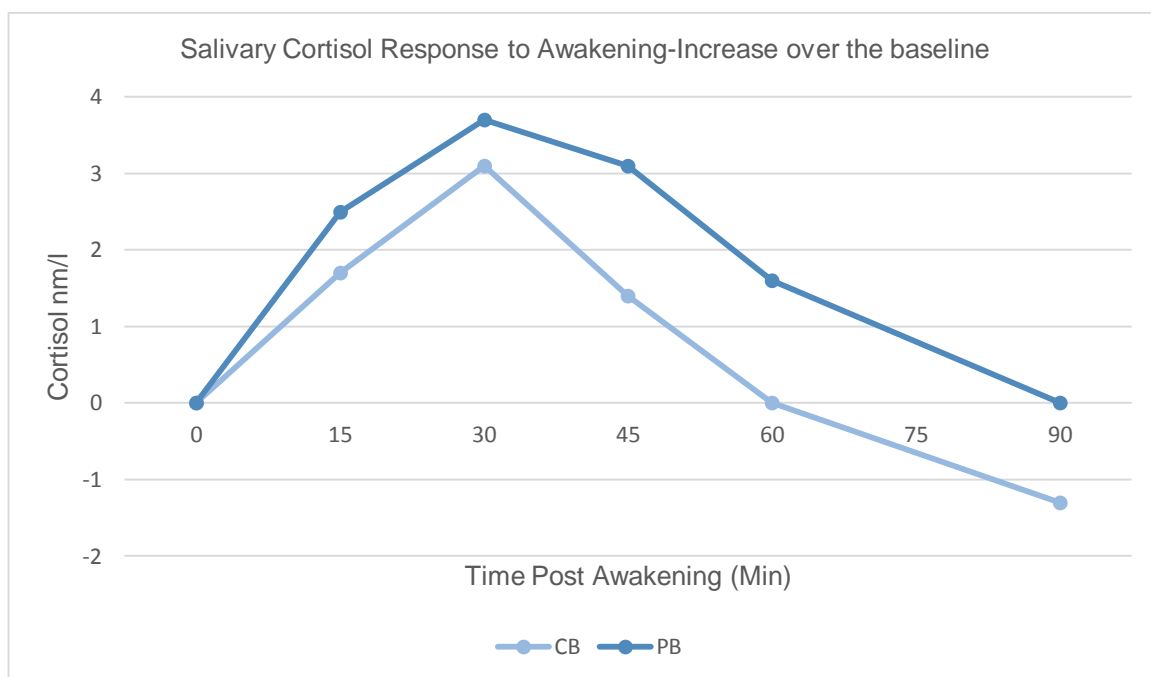


Figure 3-15 Salivary cortisol response to awakening-Increase over the base line in healthy controls without a history of childhood trauma and MDD patients with a history of childhood trauma (CB compared with PB)

3.2.13.7 Comparing AUCg and AUCi between healthy controls without a history of childhood trauma and MDD patients with a history of childhood trauma (CB compared with PA)

There were significant differences between healthy controls without a history of childhood trauma and MDD patients with a history of childhood trauma. Both AUCg and AUCi were higher in the PA group.

(AUCg60-p=0.004; AUCg90-p=0.007, AUCg60-z=-2.8; AUCg90-z=-2.7, Mean Rank AUCg60=44.7; Mean Rank AUCg90=44.2; AUCi60-p=0.02; AUCi90-p=0.007; AUCi60-z=-2.2; AUCi90-z=-2.0; Mean Rank AUCi60=42.6; Mean Rank AUCi90=42.2) Table 3.31

Table 3.31 Comparing AUCg and AUCi between healthy controls without a history of childhood trauma and MDD patients with a history of childhood trauma (CB compared with PA)

	CB		PA		p
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	
AUCg60	606.7,(511)	750±408.85	1072,(398)	991.4±361.2	0.004
AUCi60	81.0,(216)	93.5±307	220.1,(411)	224.9±354.9	0.02
AUCg90	888.0,(697)	1068.1±569.7	1431,(743)	1412.6±570.3	0.007
AUCi90	217.5,(421)	276.4±537.8	519,(708)	521.1±625.2	0.04

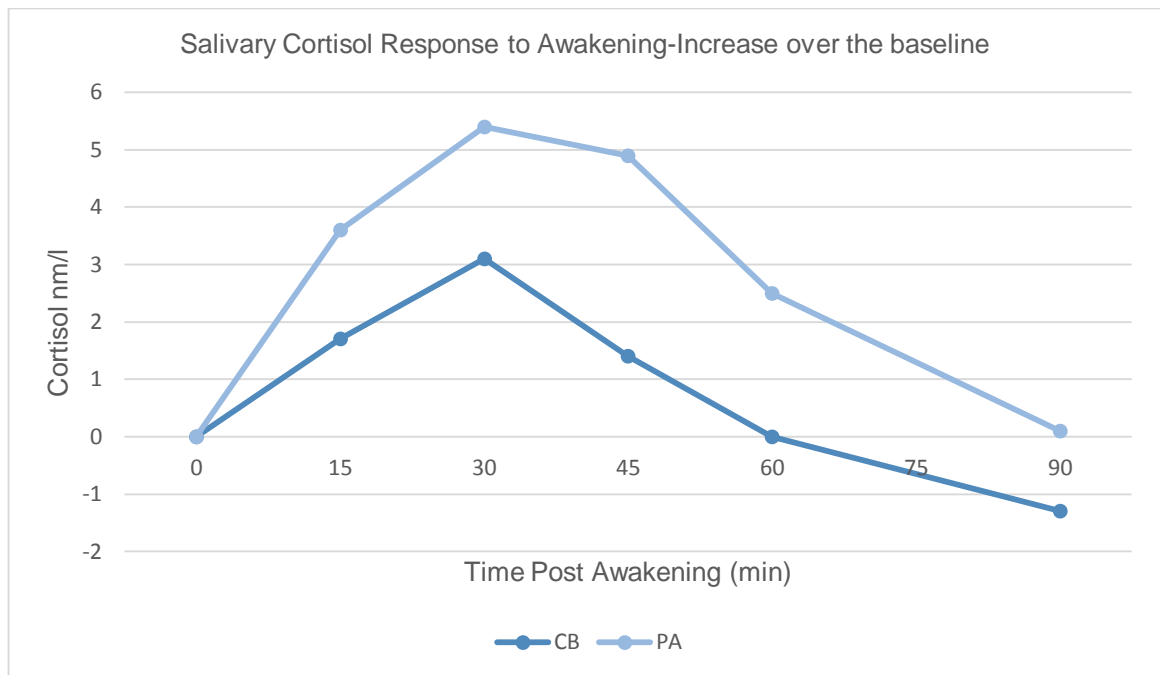


Figure 3-16 Salivary cortisol response to awakening-Increase over the baseline in healthy controls without a history of childhood trauma and MDD patients with a history of childhood trauma (CB compared with PA)

3.2.13.8 Comparing AUCg and AUCi among all the groups

AUCg and AUCi were not similar among the four groups. The PA group showed the highest post awakening increase in cortisol over baseline. Using a Kruskal Wallis test across all four groups showed that there was a significant difference for both AUCg 60-p=0.02 and AUCg 90-p=0.04 among the 4 group (Table 3.32).

Table 3.32 Comparing AUCg and AUCi among all the groups

	CB		CA		PB		PA		P
AUCg60	606.7	750±408.85	721.8	768.9±402.7	819.7	766.6±314.2	1072	991.4±361.2	0.02
AUCi60	81.0	93.5±307	34.1	98.8±310.1	74.2	151.4±228.7	220.1	224.9±354.9	0.1
AUCg90	888.0	1068.1±569.7	993.7	1089.6±565.4	1112.2	1086.5±469.5	1431	1412.6±570.3	0.04
AUCi90	217.5	276.4±537.8	197.6	290.4±529.6	171	358.3±417.6	519	521.1±625.2	0.1

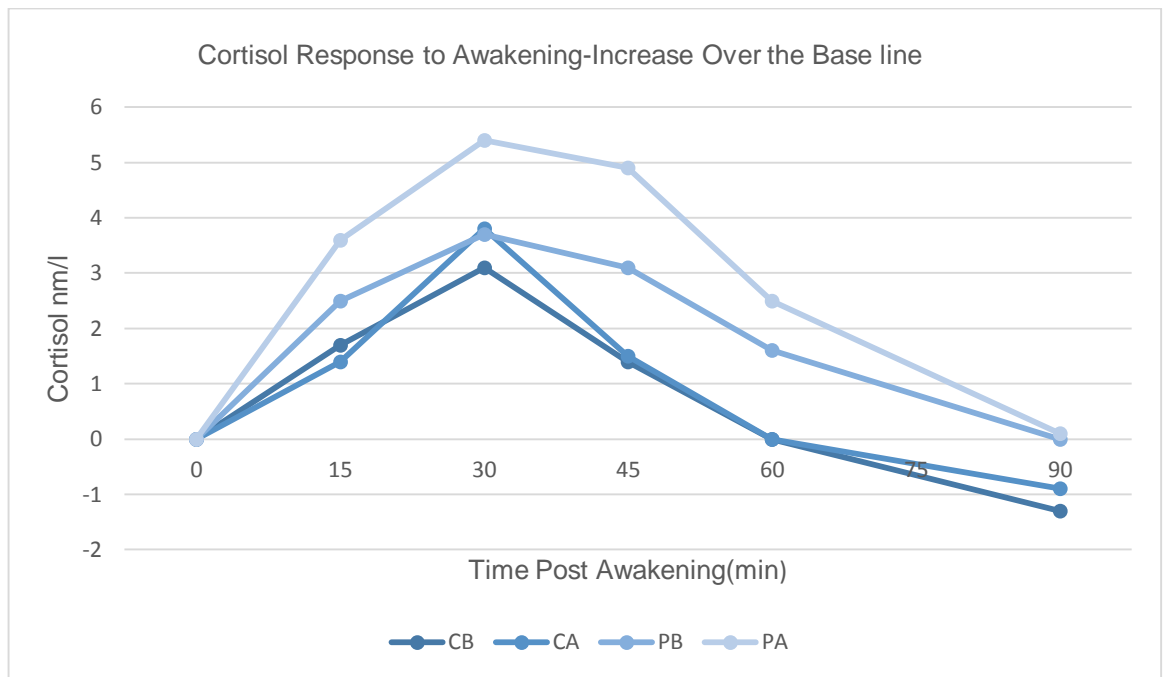


Figure 3-17 Salivary cortisol response to awakening-Increase over the base line among all the groups

3.2.14 Self-Rated Questionnaires

The questionnaires were sent to the subjects and were filled by them. A number of subjects did not fill in the forms appropriately, and therefore those questionnaires could not be used in the analysis; where possible the data for these patients were gathered from previous studies.

The scores were compared between groups, and the correlation between cortisol values--or CAR--and the scores were calculated for the different groups. If the data were normally distributed, a one way ANOVA was performed to compare the groups. If the data were not normally distributed Mann Whitney U and Kruskal Wallis tests were conducted.

Due to missing or incomplete data the numbers analyzed for these comparisons varied and are given separately for each questionnaire.

3.2.14.1 Work and Social Adjustment Scale (WSAS)

The WSAS was higher in the PA group compared with the other groups (Table 3.33). The change was significant across the 4 groups using ANOVA ($p < 0.001$).

In detail, 37 CB, 24 CA, 11 PB, 23 PA were compared to each other using one-way ANOVA and post hoc test. There was a significant difference between PA compared with CB ($p < 0.001$, mean dif = 26.3), PA compared with CA ($p < 0.001$, mean dif = 26.1), PB compared with CB ($p < 0.001$, mean dif = 22.6); and PB compared with CA ($p < 0.001$, mean dif = 22.5). The difference between the two healthy controls groups (CB compared with CA) was not significant ($p = 1.0$, mean dif = 1.25). The two patient groups did not show a significant difference (PA compared with PB, $p = 0.3$, mean dif = 0.1).

In all subjects, there were significant positive correlations at 45 and 60 minutes after awakening between WSAS scores and the cortisol (cortisol0- $p = 0.8$, $r = 0.01$; and cortisol15- $p = 0.1$, $r = 0.1$; and cortisol30- $p = 0.1$, and $r = 0.1$; cortisol45- $p = 0.003$, $r = 0.3$; cortisol60- $p = 0.01$, $r = 0.2$; cortisol90- $p = 0.07$, $r = 0.1$). In line with this, the correlation between AUC and WSAS was also positive and significant (AUCg60- $p = 0.01$; and AUCg90- $p = 0.01$; AUCg60- $r = 0.2$; AUCg90- $r = 0.2$; AUCi60- $p = 0.01$; AUCi90- $p = 0.02$; AUCi60- $r = 0.2$; AUCi90- $r = 0.2$). Table 3.34

Table 3.33 WSAS

WSAS	CB	CA	PB	PA
Median, (IQR)	0.0, (0.0)	0.0, (0.0)	19, (13)	28, (16)
Mean \pm SD	0.0 \pm 0.0	0.1 \pm 0.6	22.6 \pm 8.9	26.3 \pm 10
Stat	$P < 0.001$			

Table 3.34 Correlation between WSAS and AUC

WSAA/AUC	AUCg60	AUCi60	AUCi90	AUCg90
Stat	p=0.01 r=0..2	p=0.01 r=0.2	p=0.02 r=0.2	p 0.01 r=0.2

3.2.14.2 Zung self-rating anxiety scale (Zung)

Considering the data that we gathered from anxiety questionnaires (37 CB, 24 CA, 3 PB, 15 PA), we did not perform a comparison among the 4 groups. We compared (Mann-Whitney U test) patients and controls, and abused and non-abused subjects, as there was too much missing data for a 4 group analysis.

There was a significant difference between patients and controls, with higher scores for the patients ($p < 0.001$, $z = -5.4$, Mean Rank patients = 65.6 and Mean Rank controls = 32.4). Between abused and non-abused subjects the score was significantly different, with higher scores with abused ones ($p = 0.004$, $z = -2.9$, Mean Rank abused = 47.4, Mean Rank non-abused = 32.4). Table 3.35

In all subjects, there was a significant positive correlation between Zung self-rating anxiety scale and cortisol at 45, 60 and 90 minutes post-awakening (cortisol0- $p = 0.1$, $r = 0.1$; and cortisol15- $p = 0.08$, $r = 0.1$; and cortisol30- $p = 0.07$, $r = 0.2$, cortisol45- $p = 0.003$, $r = 0.3$; cortisol60- $p = 0.007$, $r = 0.1$; cortisol90- $p = 0.02$, $r = 0.2$).

In line with this, the correlation between AUCg and ZSAS was positive and significant (AUCg60- $p = 0.006$, $r = 0.3$; AUCg90- $p = 0.008$, $r = 0.2$).

Table 3.35 ZUNG

ZUNG	Controls	Patients	Abused	Non-abused
Stat	<p>$p < 0.001$ $z = -5.4$ mean rank patients/controls = 65.6/32.4</p>		<p>$p = 0.004$ $z = -2.9$ mean rank Ab/Nab = 47.4/32.4</p>	

Table 3.36 ZUNG correlation with cortisol and AUC

	AUCg60		AUCi60		AUCi90	AUCg90
Stat	p=0.006 r=0.3		p=0.4 r=0.9		p=0.2 r=0.1	p=0.008 r=0.2
	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
Stat	p=0.1 r=0.1	p=0.08 r= 0.1	p=0.07 r=0.2	p=0.003 r=0.3	p=0.007 r=0.1	p=0.02 r=0.2

3.2.14.3 Medical Outcomes Survey Short Forms 36 (SF-36)

Due to the data that we gathered from SF-36 questionnaires (37 CB, 24 CA, 16 PB, 3 PA), we did not make a comparison among the 4 groups. We compared (Mann-Whitney U test) patients and controls, and abused and non-abused subjects, as there were too many data missing for a 4 group analysis. The questionnaire consists of several subscales: General health (GH), Physical functioning (PF), Role limitation due to physical Health (RL-PH), Role limitation due to emotional problem (RL-E), Fatigue/Energy (FE), Emotional well-being (EW), social functioning (SF) and pain. There was a significant difference between patients and controls, and abused and non-abused subjects, with higher scores for controls and non-abused which suggests better quality of life for these subjects. Table 3.37

Table 3.37 SF36

	Patients		Controls		Statistics
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	p , z
GH	40,(45)	46.2± 24.3	80,(20)	78.4±14.7	p<0.001, z=-4.7
PF	70,(55)	62.0±23.5	100,(10)	88.5 ± 23.5	p<0.001, z=-4.1
RL-PH	25,(75)	82.0±225.1	100,(0)	91.0±22.7	p<0.001, z=-4.7
RL-E	0,(0)	10.8±27.6	100,(0)	92.5±22.9	p<0.001, z=-7.2
FE	15,(25)	22.8±18.2	70,(30)	70±17.4	p<0.001, z=-6.1
EW	84,(18)	33.4±22.3	84,(18)	82.1±14.4	p<0.001, z=-6.1
SF	37.5,(37.5)	41.7±26.8	100,(12.5)	90.1±19.7	p<0.001, z=-6.0
Pain	62.0,(57.5)	56.0±29.3	90,(20)	89.3±13.8	p<0.001, z=-5.9
	Abused		Non abused		Statistics
	Median,(IQR)	Mean±SD	Median,(IQR)	Mean±SD	p , z
GH	70,(35)	65.6±25.3	80,(20)	76.2±16.7	p=0.1, z= -1.6
PF	90,(37.5)	77.2±28.7	100,(10)	87.4±25.2	p=0.03, z=-2.0
RL-PH	100,(75)	88.1±151.3	100,(0)	89.7±27.3	p=0.007, z=-2.7
RL-E	100,(100)	58.2±47.1	100,(0)	88.8±29.9	p=0.001, z=-3.4
FE	55,(47.5)	52.4±29.9	70,(30)	65.6±21.2	p=0.04, z=-2.0
EW	76,(44)	62.3±30.8	84,(24)	79.2±17.2	p=0.01, z=-2.3
SF	75,(62.5)	68.7±32.8	100,(12.5)	89.1±22.2	p= 0.003, z=-2.9
Pain	84,(32.5)	76.5±26.4	90,(20)	86.5±18.5	p= 0.08, z=-1.7

In all subjects, there was a negative correlation between the SF-36 scores and cortisol values at several time points (Table 3.38). The correlation between GH at cortisol30 (p=0.02), cortisol45 (p=0.01), and cortisol60 (p=0.01) and cortisol90 (p=0.007) was significant. The correlation between PF and cortisol was negative and insignificant; the same as RL-PH (Table 3.38).

The correlation between RL-E was significant at all-time points except time of awakening (cortisol0-p=0.3, cortisol15-p=0.001, cortisol30-p=0.005, cortisol45-p=0.001, cortisol60-p<0.001, cortisol90-p=0.004). The correlation between FE and cortisol was significant and negative at 45 (p=0.008), 60(p=0.004) and 90 (p=0.02) minutes post awakening. The correlation between EW and cortisol was significant at all-time points except at awakening (cortisol0-p=0.1, cortisol15-p=0.02, cortisol30-p=0.01, cortisol45-p=0.004, cortisol60-p=0.003, cortisol90-p=0.02). The correlation between SF and cortisol was not significant at any time points (Table 3.38). The correlation between pain and cortisol was significant at all time points except at time of awakening (cortisol0-p=0.1, cortisol15-p=0.001, cortisol30-p=0.001, cortisol45-p<0.001, cortisol60-p<0.001, cortisol90-p=0.001) (Table 3.38).

In line with these results, there was a negative correlation between AUCg60 and SF-36. This correlation was significant for GH, RL-E, EW and pain (Table 3.38). AUCi60 revealed a significant correlation with SF-36 which was significant at RL-E, FE and pain (Table 3.38). In agreement with these results, there was a negative correlation between AUCg90 and SF-36 which was significant at GH, RL-E, FE, EW and pain (Table 3.38) AUCi90 showed a significant correlation at RL-E, FE, EW and pain which was negative at all sections (Table 3.38).

Table 3.38 SF-36 Correlation with cortisol and AUC

	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90	
GH	p=0.2 r=-0.1	p=0.07 r=-0.2	p=0.02 r=-0.2	p=0.01 r=-0.2	p=0.01 r=-0.2	p=0.007 r=-0.3	
PF	p=0.8 r=-0.01	p=0.3 r=-0.09	p=0.2 r=-0.1	p=0.08 r=-0.1	p=0.2 r=-0.1	p=0.2 r=-0.1	
RL-PH	p=0.8 r=0.01	p=0.3 r=-0.1	p=0.2 r=-0.1	p=0.1 r=-0.1	p=0.1 r=-0.1	p=0.1 r=-0.1	
RL-E	p=0.3 r=-0.1	p<0.001 r=-0.2	p=0.005 r=-0.3	P<0.001 r=-0.3	p<0.001 r=-0.3	p=0.004 r=-0.3	
FE	p=0.7 r=-0.03	p=0.2 r=-0.1	p=0.05 r=-0.2	p=0.008 r=-0.2	p=0.004 r=-0.3	p=0.02 r=-0.2	
EW	p=0.1 r=-0.03	p=0.02 r=-0.1	p=0.01 r=-0.2	p=0.004 r=-0.2	p=0.003 r=-0.3	p=0.02 r=-0.2	
SF	p=0.6 r=-0.05	p=0.08 r=-0.1	p=0.1 r=-0.1	p=0.08 r=-0.1	p=0.06 r=-0.2	p=0.08 r=-0.1	
Pain	p=0.1 r=-0.1	P<0.001 r=-0.3	P<0.001 r=-0.3	p<0.001 r=-0.3	p<0.001 r=-.04	p=0.001 r=-0.3	
		AUCg60		AUCi60		AUCg90	AUCi90
GH		p=0.02 r=-0.2		p=0.2 r=-0.1		p=0.01 r=-0.2	p=0.05 r=-0.2
PF		p=0.2 r=-0.1		p=0.2 r=-0.1		p=0.2 r=-0.1	p=0.2 r=-0.1
RL-PH		p=0.2 r=-0.1		p=0.1 r=-0.1		p=0.2 r=-0.1	p=0.1 r=-0.1
RL-E		p=0.003 r=-0.3		p=0.008 r=-0.2		p=0.001 r=-0.3	p=0.005 r=-0.3
FE		p=0.4 r=-0.2		p=0.009 r=-0.2		p=0.02 r=-0.2	p=0.006 r=-0.3
EW		p=0.006 r=-0.3		p=0.05 r=-0.2		p=0.006 r=-0.3	p=0.04 r=-0.2
SF		p=0.1 r=-0.1		p=0.1 r=-0.1		p=0.09 r=-0.1	p=0.1 r=-0.1
Pain		p<0.001 r=-0.3		P<0.001 r=-0.3		p<0.001 r=-0.3	p<0.001 r=-0.3

3.2.14.4 Inventory of Depressive Symptomatology (IDS)

Due to the data that we gathered from IDS questionnaires (37 CB, 24 CA, 3 PB, 12 PA), we did not make a comparison among the 4 groups. Instead, we

compared (Mann-Whitney U test) patients and controls, and abused and non-abused subjects, as there were too many missing data for a 4 group analysis.

There was a significant difference between MDD patients and controls, with higher scores in the patient group ($p<0.001$, $z=-5.7$, Mean Rank =67.9).

There was a significant difference between abused and non-abused subjects, with high scores in abused subjects ($p=0.02$, $z=-2.3$, Mean Rank=44.5).

In all subjects, the correlation between IDS and cortisol values was significant at all time points except at time of awakening and 90 minutes post awakening (cortisol0- $p=0.06$, and $r=0.2$; cortisol15- $p=0.01$, $r=0.2$; cortisol30- $p=0.006$, $r=0.3$; cortisol45- $p=0.01$, $r=0.2$; cortisol60- $p=0.01$, $r=0.2$; cortisol90- $p=0.1$, $r=0.1$).

In line with this, there was a significant positive correlation between AUCg60, 90 and IDS (AUCg60- $p=0.005$; AUCg90- $p=0.01$; AUCg60- $r=0.3$; AUCg90- $r=0.2$).

Table 3.39 IDS

	Controls	Patients	Abused	Non-abused
Stat	$p<0.001$ $z=-5.7$ mean rank patients/controls =67.9/31.2		$p=0.02$ $z=-2.3$ mean rank Ab/Nab=44.5/32.7	

Table 3.40 IDS correlation with cortisol and AUC

	AUCg60		AUCi60		AUCi90	AUCg90
Stat	$p=0.005$ $r=0.3$		$p=0.1$ $r=0.1$		$p=0.1$ $r=0.1$	$p=0.01$ $r=0.2$
	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
Stat	$p=0.06$ $r=0.2$	$p=0.01$ $r=0.2$	$p=0.006$ $r=0.3$	$p=0.01$ $r=0.2$	$p=0.01$ $r=0.2$	$p=0.1$ $r=0.1$

3.2.14.5 Oslo Social Support scale (OSS-3)

Thirty-seven CB, 24 CA, 11PB and 24PA were analyzed. There was no difference among the 4 groups in terms of total score ($p=0.07$, $df=3$). Table 3.41

In all subjects, there was not a significant correlation at any time point (cortisol0-p=0.1, and r=-0.1; cortisol15-p=0.3, and r=-0.9; cortisol30-p=0.3, and r=-0.9; cortisol45-p=0.1, r=-0.1; cortisol60-p=0.2, r=-0.1; cortisol90-p=0.2, r=-0.1)

In line with this, AUC did not reveal a significant correlation with OSS-3 (AUCg60-p= 0.2; and AUCg90-p=0.3; AUCg60-r=-0.1; AUCg90-r=-.09; AUCi60-p=0.6; AUCi90-p=0.7, AUCi60-r=0.04; AUCi90-r= 0.03). Table 3.42

Table 3.41 OSS-3

	CB	CA	PB	PA
Stat	p= 0.07 , df=3			

Table 3.42 OSS-3 correlation with cortisol and AUC

	AUCg60		AUCi60		AUCi90	AUCg90
Stat	p=0.2 r=-0.1		p=0.6 r=0.04		p=0.7 r=0.03	p=0.3 r=-0.9
	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
Stat	p=0.1 r=-0.1	p=0.3 r=-0.9	p=0.3 r=-0.9	p=0.1 r=-0.1	p=0.2 r=-0.1	p=0.2 r=-0.1

3.2.14.6 List of Threatening Events (LTE)

Thirty seven CB, 24 CA, 12 PB and 25 PA were analyzed. There was a significant difference among the 4 groups in the overall score of the LTE questionnaire (p<0.001, df=3). The PB group showed the highest scores (mean=1±0.8, median=1(IQR=0.7), Mean Rank=71.9).

In all subjects, there was no significant correlation between LTE and cortisol at any time point (cortisol0-p=0.7, and r=-0.03; cortisol15-p=0.7, r=0.02; cortisol30-p=0.8, r=0.01; cortisol45-p=0.5, and r=0.06; cortisol60-p=0.6, r=0.04; cortisol90-p=0.8, r=-0.02).Table 3.44

In line with this, there were no significant correlations between AUC and LTE (AUCg 60-p=0.7; AUCg90-p=0.8; AUCg60-r=0.03; AUCg90-r=0.02; AUCi60-p=0.2; AUCi90-p=0.3, AUCi60-r=0.1; AUCi90-r=0.09) Table 3.44

Table 3.43 LTE

	CB	CA	PB	PA
Stat	P<0.001 , df= 3			

Table 3.44 LTE correlation with cortisol and AUC

	AUCg60		AUCi60		AUCi90	AUCg90
Stat	p=0.7 r= 0.03		p=0.2 r= 0.1		p=0.3 r= 0.09	p=0.8 r=0.1
	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
Stat	p=0.7 r=-.03	p=0.7 r=0.02	p=0.8 r=0.01	p=0.5 r=0.06	p=0.6 r=0.04	p=0.8 r=-0.02

3.2.14.7 Impact of Event Scale (IES)

Due to missing data (16 CB, 15 CA, 1PB and 1PA) we only analyzed the controls and not patients. There was no significant difference between abused controls and non-abused controls (p=0.7, z=-.0.2, Mean Rank=16.4).

The correlation between cortisol at all-time points in all subjects, and the IES scores was not significant (cortisol0-p=0.8, r=-0.04; cortisol15-p=0.4, r=-0.1; cortisol30-p=0.6, r=-0.07; cortisol-45-p=0.5, r=-0.09; cortisol60-p=0.7, r=-0.06; cortisol90-p=0.8, r=-0.02).

In line with this, the correlation between AUC and IES was not significant (AUCg 60-p=0.7; AUCg90-p=0.6; AUCg60-r=-0.06; AUCg90-r=0.08; and AUCi60-p= 0.8 AUCi90-p=0.9, AUCi60-r=-0.02; AUCi90-r=0.01).

Table 3.45 IES correlation with cortisol and AUC

	AUCg60		AUCi60		AUCi90	AUCg90
Stat	p=0.7 r=- 0.06		p=0.2 r=- 0.02		p=0.3 r= -0.01	p=0.6 r= -0.08
	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
Stat	p=0.8 r=-.04	p=0.4 r=-0.1	p=0.6 r=-.07	p=0.5 r=-0.09	p=0.7 r=-0.06	p=0.8 r=-0.02

3.2.14.8 Perceived Stress Scale (PSS)

16 CB and 13 CA were analyzed. There was no difference between CA and CB ($p=0.6$, $t=-0.4$, $F=1.6$, $df=27$) (mean =23.2± 5.8, median=22(IQR=7.5)). There was no data in PB group, and only 3 subjects in the PA had scores available for this variable.

In all subjects, the correlation between cortisol and PSS scores was not significant at any time points (cortisol0- $p=0.9$, $r=-0.1$; cortisol15- $p=0.5$, $r=-0.1$; cortisol30- $p=0.3$, $r=-0.1$; cortisol45- $p=0.3$, $r=-0.1$; and cortisol60- $p=0.4$, $r=-0.1$; cortisol90- $p=0.5$, $r=-0.1$).

In agreement with this, the correlation between AUC and PSS was not significant (AUCg60- $p=0.5$; AUCg90- $p=0.5$; AUCg60- $r=-0.1$; AUCg90- $r=-0.1$; AUCi60- $p=0.3$; AUCi90- $p=0.2$, AUCi60- $r=-0.1$; AUCi90- $r=-0.1$).

Table 3.46 PSS correlation with cortisol and AUC

	AUCg60		AUCi60		AUCi90	AUCg90
Stat	p=0.5 r=-0.1		p=0.3 r=-0.1		p=0.2 r=-0.1	p=0.5 r=-0.1
	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
Stat	p=0.9 r=-0.1	p=0.5 r=-0.1	p=0.30 r=-0.1	p=0.3 r= -0.1	p=0.4 r=-0.1	p=0.5 r=-0.1

3.2.14.9 Pittsburgh Sleep Quality Index (PSQI)

15 CB and 13 CA were analyzed. There was no data in PB group. One subject in the PA group returned the questionnaire. There was no difference between CA and CB (Table 3.47).

Table 3.47 PSQI

	Quality	Latency	Duration	Dysfunction	Efficiency	Disturbance	Medication
Statistics	p=0.5 z=-0.5	p=0.03 z=-0.2	p=0.8 z=-0.1	p=0.6 z=-0.4	p=0.8 z=-0.1	P=0.6 Z=-0.3	p=0.9 z=-.00
Mean±SD							
CB	0.6±0.6	1.2±0.8	0.8±0.8	0.3±0.4	0.2 ± 0.4	1.1±0.6	0.4±1.0
CA	0.6±0.9	0.5±0.5	1.0±1.0	0.3±0.6	0.4 ± 0.9	1.1±0.6	0.3±0.6
Median,(QIR)							
CB	1,(1)	1,(1)	1,(1)	0.0,(1)	0,(1)	1,(0)	0,(0)
CA	0,(1)	1,(1)	1,(2)	0.0,(1)	0,(1)	1,(1)	0,(1)

The correlation between PSQI and cortisol at all-time points was not significant (Table 3.48). In line with this, the correlation between PSQI and AUC was not significant (Table 3.48).

Table 3.48 PSQI correlation with cortisol and AUC

	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
Quality	p=0.4 r=0.1	p=0.2 r=0.2	p=0.6 r=0.08	p=0.5 r=0.1	p=0.8 r=0.04	p=0.8 r=0.02
Latency	p=0.6 r=-0.08	p=0.5 r=-0.1	p=0.3 r=-0.1	p=0.6 r=-0.07	p=0.1 r=-0.1	p=0.4 r=-0.1
Duration	p=0.7 r=-0.05	p=0.6 r=-0.08	p=0.2 r=-0.2	p=0.2 r=-0.2	p=0.4 r=-0.1	p=0.6 r=0.08
Dysfunction	p=0.4 r=-0.1	p=0.09 r=-0.3	p=0.5 r=-0.1	p=0.4 r=-0.1	p=0.3 r=-0.1	p=0.5 r=-0.1
Efficiency	p=0.3 r=0.1	p=0.8 r=0.03	p=0.8 r=0.03	p=0.6 r=0.08	p=0.4 r=0.1	p=0.4 r=0.1
Disturbance	p=0.9 r=0.02	p=0.4 r=-0.1	p=0.1 r=-0.3	p=0.1 r=-0.2	p=0.4 r=-0.1	p=0.5 r=-0.1
Medication	p=0.3 r=-0.1	p=0.9 r=-0.1	p=0.5 r=-0.1	p=0.9 r=-0.02	p=0.7 r=-0.05	p=0.3 r=-0.1
	AUCg60	AUCi90	AUCg90	AUCi90		
Quality	p=0.5, r=0.1	p=0.8 r=0.02	p=0.6 r=0.08	p=0.9 r=-0.01		
Latency	p=0.3 r=-0.1	p=0.7 r=-0.05	p=0.3 r=-0.1	p=0.6 r=-0.08		
Duration	p=0.3 r=0.1	p=0.3 r=-0.1	p=0.3 r=-0.1	p=0.4 r=-0.1		
Dysfunction	p=0.3 r=0.1	p=0.7 r=0.07	p=0.3 r=0.1	p=0.6 r=0.08		
Efficiency	p=0.8 r=0.1	p=0.4 r=-0.1	p=0.7 r=0.06	p=0.5 r=-0.1		
Disturbance	p=0.3 r=-0.1	p=0.08 r=-0.3	p=0.3 r=-0.1	p=0.1 r=-0.2		
Medication	p=0.8 r=0.04	p=0.2 r=0.2	p=0.6 r=-0.08	p=0.2 r=0.2		

3.2.14.10 Correlation between specific type of abuse and all individual time points of cortisol

There was a positive correlation between the severity of certain type of abuse and cortisol at all-time points in all subjects (Table 3.49).

There was a significant correlation between emotional abuse severity and cortisol at 15 ($p=0.01$), 30 ($p=0.003$), 45 ($p=0.003$) and 60 ($p=0.02$) minutes post-awakening (Table 3.49). There was a significant correlation between physical abuse severity and cortisol at time of awakening ($p=0.04$).

There was a significant correlation between severity of sexual abuse and cortisol at 30($p=0.02$), 45($p=0.006$), 60($p=0.007$) and 90($p=0.02$) minutes post-awakening. There was no significant correlation between emotional and physical neglect and cortisol at any time point, except at 15 minutes after awakening in emotional neglect (Table 3.49).

Table 3.49 Correlation between child abuse and cortisol at all individual time points

	cortisol0	cortisol15	cortisol30	cortisol45	cortisol60	cortisol90
Emotional	p=0.3 r=0.8	p=0.01 r=0.2	p=0.003 r=0.2	p=0.003 r=0.2	p=0.02 r=0.2	p=0.2 r=0.1
Physical	p=0.04 r=0.1	p=0.2 r=0.1	p=0.3 r=0.09	p=0.2 r=0.1	p=0.3 r=0.09	p=0.2 r=0.1
Sexual	p=0.3 r=0.08	p=0.1 r=0.1	p=0.02 r=0.2	p=0.006 r=0.2	p=0.007 r=0.2	p=0.02 r=0.2
Emotional n	p=0.4 r=0.08	p=0.03 r=0.2	p=0.08 r=0.1	p=0.08 r=0.1	p=0.2 r=0.1	p=0.8 r=0.2
Physical n	p=0.4 r=0.07	p=0.1 r=0.1	p=0.3 r=0.09	p=0.2 r=0.1	p=0.5 r=0.06	p=0.6 r=0.04

3.2.14.10.1 Correlation between specific type of abuse and the score of HAM-D 17, 21

There was a positive and significant correlation between the score of HAM-D 17, 21 and the severity of all types of abuse except physical abuse and physical neglect .Table 3.50

Table 3.50 Correlation between child abuse and HAM-D 17, 21

	Emotional	Physical	Sexual	Emotional n	Physical n
HAM-D 17	p<0.001 r=0.3	p=0.2 r=-0.1	p=0.02 r=0.2	p=0.001 r=0.2	p=0.1 r=0.1
HAM-D 21	p<0.001 r=0.3	p=0.3 r=-0.09	p=0.02 r=0.2	p=0.001 r=0.3	p=0.1 r=0.1

Chapter 4 DISCUSSION

4.1 Overview

With the fast increasing population of people who become depressed, and considering the adverse consequences of MDD, which could affect several aspects of the lives of people and their societies, it is time to consider this serious and debilitating disorder as a global concern.

The amount of extensive research that has been conducted to understand the complexity of MDD has shown that gaining insight into MDD is not easy and simple. Identifying underlying biomarkers of depression may play a significant role in solving the puzzle of MDD; thus, research into biomarkers could be a big step towards decreasing the damaging consequences of depression.

In order to identify biomarkers of MDD, numerous studies have been conducted on several types of biomarkers. In my thesis I focused on a number of potential biomarkers of MDD, consisting of the BDNF val66met polymorphism, amygdala volume and the CAR as an index of the HPA axis combined with early life stress, as explained in detail in the previous chapters.

In brief, our first study did not find any modulatory effects of the val66met polymorphism on the grey matter volume of right and left amygdala. In the second study, we showed that the CAR was most elevated in those who were both depressed and had a history of ELS, which supports the argument that the effects of early life stress and MDD on the HPA axis may be additive. This is a novel result in the literature.

4.2 The first study

Our study aimed to find a link between the val66met BDNF polymorphism and the grey matter of the amygdala. This study did not focus on other brain structures, although, it was a part of a wider study which did investigate the

modulatory effect of val66met BDNF polymorphism on the grey matter volume of several other areas of the brain (see Appendix A)

4.2.1 Structure of the Brain, Amygdala

4.2.1.1 Healthy controls and MDD Patients

Amygdala volume was compared between healthy controls and MDD patients; our study did not find a reduction of amygdala volume in MDD patients. This result is in agreement with an early meta-analysis that found no changes in amygdala volume associated with depression (Campbell et al., 2004). In association with this, our result is consistent with a 1 year follow-up study by Frodl et al. (2004) which was conducted on 30 MDD patients (HAM-D17=23.7±6.9; and age=18-65 years; and mean±SD age=48.4±13.4 years; and mean±SD duration of illness=9.1±10.2) consisting of 11 patients with first episode of depression and 19 patients with recurrent depressive disorder. They matched 30 healthy controls in terms of age (20-65 years, and mean±SD age=45.7±12.9), gender and the tendency to use either the right or left hand. All the subjects were tested after 1 year. Remission was defined as HAM-D 17 score ≤ 7. No changes in amygdala volume were observed either in remitted or non-remitted MDD patients with first episode of depression or recurrent depressive disorder. As explained before our patients were in their acute phase of third depressive episode and they had a history of recurrent depression.

Another study that did not find significant changes on amygdala volume was conducted on 34 drug-resistant MDD patients compared to 17 age-matched healthy control subjects (Mervaala et al., 2000). Although our subjects had a history of recurrent depression rather than a more chronic, treatment-resistant type of depression, this result was consistent with our result.

In contrast, in a more recent meta-analysis, Sacher et al. (2012) found lower grey matter amygdala volume in MDD patients, a result which is different to our result. This latter meta-analysis consisted of VBM and MRI studies that investigated structural changes, as well as functional (18F)-fluorodeoxyglucose Positron Emission Tomography (FDG-PET) studies, designed to show changes in glucose-metabolism at rest; whereas, we did not use VBM or FDG-PET ; In our study , instead magnetisation-prepared rapid gradient echo (MP-RAGE) T1-weighted scans were acquired at 1.5 T and the MP-RAGE volume acquired using the Alzheimer's disease Neuroimaging Initiative (ADNI) custom pulse sequence. In association with this, related to treatment of MDD, the previous meta-analysis by Hamilton et al. (2008) found that the amygdala volume was decreased in un-medicated MDD patients and increased in MDD patients who received medication. Although, most of our MDD patients were taking antidepressant medication, no amygdala volume changes were observed in our study which is not in agreement with this meta-analysis. The inconsistency between the results could be due to the chronicity of MDD of the subjects, as our participants had a history of recurrent depression characterized by discrete acute depressive episodes with periods of euthymia rather than a more chronic, treatment-resistant type of depression; the other issue could be due to the way that the amygdala was characterized for the volume estimation ; because although they mentioned that they included studies in which the amygdala was defined independent of the hippocampus, the definitions of amygdala volume reported in the larger literature were highly variable and not easily quantifiable, thus the impact of variation in amygdala boundary definition could lead to this inconsistency ; and the last factor should be considered is that the characteristic of the subjects in terms of age,

gender and the proportion of medicated or un-medicated patients were not similar between our study and theirs (Hamilton et al., 2008).

In line with these results, Eijndhoven et al. (2009) conducted a study on 20 MDD patients with a current first episode of depression (age=18-56 years; mean age \pm SD=34.1 \pm 11.6 years), 20 MDD patients without medication who had recovered from a first episode (age=18-53 years; mean age \pm SD=35.8 \pm 11.6 years), and 20 healthy controls (age=18-53 years; mean age \pm SD=37.3 \pm 12.7 years). They found a heightened amygdala volume in first episode MDD patients compared with the other groups. It was argued that the enlargement of amygdala volume was a state marker of depression, and it was not associated with any medication used by MDD patients. This result was also in agreement with a study by Frodl et al. (2003) conducted on 30 inpatients with a first episode of depression and 27 inpatients with recurrent major depression, together with healthy controls from the local community matched for age, gender, and the tendency to use either right or left hand. Enlargement of amygdala volume was observed only in patients in the first episode of depression compared with patients with recurrent major depression and healthy control subjects. Our subjects were not in their first episode of illness, as our participants had a history of recurrent depression characterized by acute depressive episodes with periods of euthymia, and this could be an explanation for the inconsistency between the results, from this study and ours.

In contrast with finding of amygdala volume enlargement, decreased amygdala volume were observed in MDD patients compared with healthy controls in several other previous studies (Hasting et al., 2004; Tang et al., 2007; Frodl et al., 2008). For example, Frodl et al. (2008) investigated 38 inpatients with major depression (mean \pm SD age=46.1 \pm 11.3 years) and 30 healthy controls, matched for age

(mean \pm SD age=43.6 \pm 11.3 years), gender and the tendency to use either right or left hand. VBM volumes were estimated from magnetic resonance images; regular assessment of psychopathologic results was conducted during the inpatient phase, and then after 1, 2, and 3 years. MDD patients showed a decrease in grey matter density of the left amygdala. Most of their patients remitted after the inpatient treatment phase, but during the follow-up period, about 17 patients (45%) relapsed. The remaining 21 patients (55%) did not relapse over 3 years. No differences in grey matter amygdala volume were observed between remitted patients without relapse and remitted patients with relapse. This was a prospective, longitudinal 3 year study, which did not use similar MRI acquisition methods to ours. In agreement with this result, Tang et al. (2007) in another VBM MRI study, found decreased grey matter volume in the right amygdala in a group that consisted of 14 female MDD patients (mean age \pm SD=29.5 \pm 6.84 years; mean education \pm S.D years=11.43 \pm 2.65; and mean depressive episode duration \pm S.D months=5.44 \pm 5.22) who had a score of \geq 18 on HAM-D 17 with a first episode of major depression in comparison to 13 healthy females controls, matched for age (mean age \pm S.D years=29.46 \pm 6.86) and education (mean education \pm S.D years=12.23 \pm 3.30). A few subjects in both groups had comorbidity with general anxiety disorder. It should be considered that our subjects were not restricted to females and they were not in their first episode of their illness.

Similar to their results, decreased amygdala volume was demonstrated by Hasting et al. (2004) in MDD females compared with MDD males. Of note, several subjects had a history of childhood abuse in the female group. The result of their study could support the assertion that decreased amygdala volume may be

related to gender. However, our study did not make a comparison between males and females.

In brief, the results from these previous studies described above, are inconsistent as to the presence of changes to grey matter amygdala volume in MDD, and in regard to illness factors that may be associated with the presence of such changes such as the lack of similarity in characteristics of the subjects, such as gender and the form of MDD e.g. severity, chronicity and number of recurrences. In our study, subjects had a history of recurrent depression characterized by acute depressive episodes with periods of euthymia rather than a more chronic, treatment-resistant type of depression.

4.2.1.2 Comparing Val/Val Carriers and Met Carriers

The mean amygdala volume was analyzed in val/val genotype and met carrier subjects. The results showed that carrying the met allele did not have a significant impact on the amygdala volume.

Previous studies investigating the influence of the val66met BDNF polymorphism on amygdala volume have shown inconsistent results. Similar to our result, Forde et al. (2014) did not find an effect of met-homozygosity and heterozygosity on grey matter and white matter of the brain structure. In particular, they recruited 60 healthy controls, 20 in each genotypic group (val/val, val/met and met/met). They used VBM and surface reconstruction methods. MRI scans were acquired on a 3 T scanner. They found no evidence to show a met dose-effect on amygdala volume—from an intracellular point of view, there seems to be a met-dose effect , with increasing the number of met allele, on intracellular localization (Chen et al., 2004) and adjustment of activity-dependent secretion of BDNF (Chen et al., 2006); These effects are presumably caused by abnormal intracellular packaging and regulation of the secretion of BDNF in the presence of Met (Egan et al., 2003;

Chen et al., 2004). These abnormalities cause changes in the BDNF activity-dependent processes of human brain development and cortical plasticity. Previous studies have selected met-carriers to investigate the effects of the val66met BDNF polymorphism rather than the certain met-dose effect, probably due to an absence of met-homozygotes in the population – in any of the balanced genetic group of their subjects. This study has shown that the met-allele has a significant but non-uniform effect on the brain structure, with homozygotes (met/met and val/val) more similar than heterozygotes (val/met). Not similar to our study, their subjects consisted of healthy controls instead of both MDD and healthy controls. However, they argued that by grouping all met-carriers together, previous studies, similar to our study, may have missed an important factor in the interaction between the met and val alleles. Thus, their results presented a U-shaped profile in BDNF, where heterozygotes were not similar to both sets of the homozygotes (Forde et al., 2014).

4.2.1.2.1 Val/Val Carriers and Met Carriers in healthy controls

In order to investigate the role of the met allele in healthy controls, the amygdala volume was compared between val/val genotype and met carrier groups. There were no differences between these two groups.

Our result differ from the outcome of a study by Montag et al. (2009), who showed decreased right and left amygdala volumes, using VBM, in healthy controls who were met allele carriers. It should be noted that their subjects relied on a self-reported assessment of depression and they did not use a standardized structured interview for psychiatric diagnoses to characterise the healthy controls. Substance abuse was not considered to be an exclusion criterion for their study, which is problematic given evidence that this can affect amygdala volumes (Padula et al., 2015).

However, our results are consistent with research conducted by Frodl et al. (2007) in MDD patients and by Gerritsen et al. (2012) showing no effect on amygdala volume in healthy subjects of carrying the BDNF met allele with a history of childhood adversity ; although we didn't measure childhood adversity in our study.

Consistent with our result, in particular, Frodl et al. (2007) investigated the effect of the BDNF val66met polymorphism on amygdala volume in MDD patients and in healthy control subjects, in 120 subjects consisting of 60 patients with major depression (age=18-65 years; mean \pm SD age=44.2 \pm 11.8 years; HAM-D 17=23 \pm 6.3) and 60 healthy controls (age=22-64 years; mean \pm SD age=41.6 \pm 12.3 years). No differences in amygdala volume were observed in MDD patients compared with healthy controls. All MDD patients were inpatients. 30 patients had a first depressive episode, and 30 patients had recurrent episodes. Almost all patients, except those who were not taking antidepressants medications, were in an outpatient service and were treated with antidepressants before the current admission. MRI was conducted in the first 2 weeks after admission to the hospital. In agreement with our study, no significant changes of amygdala volume were observed.

4.2.1.2.2 Val/Val carriers and Met carriers in MDD patients

The argument about the function of the met allele in MDD was tested by comparing val/val genotype and met allele carriers in MDD patients. The finding of an insignificant reduction of amygdala volume supports the suggestion that the met allele does not play a major role in determining amygdala volume in MDD. To our knowledge, there is no previous study that has investigated the effect of carrying the met allele on grey matter amygdala volume in comparison to the val/val genotype in only MDD patients groups.

4.3 The second study

Cortisol secretory activity increases at the early morning and reaches a peak point following awakening; thereafter it falls with a decreasing trend over the remainder of 24 hours (Edwards et al., 2001), although inconsistent results of cortisol secretory activity have been previously observed in subjects experiencing MDD (Trestman et.al., 1995; Goodyer et al., 1996).

Our study found that, among all subjects (including healthy controls and patients), the peak cortisol level was observed at 30 minutes after awakening (mean \pm SD=15.3 \pm 8.2; median=15.10 nm/l); which was consistent with previous findings which observed the rises of cortisol by anywhere between 38% and 75%—of note, our study observed a 46% increase—compared to the cortisol at awakening, peaking 30-45 minutes after awakening (Clow et al., 2004; Fries et al., 2009).

4.3.1 CAR in Healthy Controls and MDD Patients

The study found a higher cortisol and AUC-CAR- in MDD patients compared with healthy controls. Thus, when all patients were combined together, the total post-awakening secretion of cortisol -AUCg- and the responsivity of the HPA axis -AUCi- were both higher in MDD. It should be noted that this overall comparison consists of both healthy controls and MDD patients who had experienced early life stress, as well as those who had not. As will be explained later, the lack of similarity between those subjects with and without a history of childhood abuse, and the extent to which it is MDD rather than childhood abuse causing the increasing in the CAR, cannot be addressed in a straightforward comparison of patients and controls. However, this result is consistent with a number of previous studies which have shown an increased CAR in MDD patients (Vreeburg et al., 2009; Ulrike et al., 2013; Dienes et al., 2013). In particular, an increased CAR was found in the following groups of MDD patients: middle aged subjects

(Vreeburg et al., 2009), adolescent girls (Ulrike et al., 2013) and adult females (Dienes et al., 2013). These prior studies findings are consistent with our results, although our subjects were both females and males with a wide age range.

In contrast, however, other studies have shown a blunted CAR in young adult women with mild to moderate depression (Stetler et al., 2005) and in older people with a 6 month depression (Rhebergen et al., 2015).

The inconsistency between the prior results, in regards to the CAR in MDD patients, could be caused by a number of issues. First of all, the severity of depression might be a potential factor; whilst most studies consisted of MDD patients who had undergone appropriate diagnostic assessment, others investigated subjects with increased depressed mood without actual diagnosis of depression. However, to our knowledge there has been no study investigating the effect of severity of depression on the CAR in MDD patients. The other issue which should be considered is that psychosocial factors associated with depression, such as work stress and financial strain, were not always considered in all studies; it is well documented that these factors may be associated with both increased vulnerability to depression and alterations in the CAR (Chida et al., 2009; Dedovic et al., 2015). In detail, Chida et al.(2009), in a meta-analysis, showed that job stress and social stress were related to an increased CAR, whereas fatigue, burnout, or exhaustion were characterized by a decreased CAR. Thus, ignoring the psychosocial factors could be another important issue contributing to the inconsistency of the results between previous studies.

4.3.2 Effects of childhood abuse

Our study did not find a significant difference in the cortisol at any time point or in AUC-CAR- when comparing abused and non-abused groups when all subjects were considered together. As explained before, such a comparison does not take

full account of the effects of depression. However, this result is inconsistent with a previous study which found a decreased CAR in subjects with a history of early life stress (Heim et al., 2001) and with a study which found a flattened CAR in mid-adulthood in association with cumulative maltreatment in childhood (Power et al., 2012).

These inconsistent results could be due to a number of reasons. One explanation could be that the studies select other methods to assess childhood adversity. The ascertainment of childhood adversities and other psychosocial maltreatments is not straightforward because all methods may be affected by biases and inconsistencies (Power et al., 2012). In particular, biases in self-reported of sexual abuse have been observed in previous research, consisting of problems such as forgetting, denial, misunderstanding, and embarrassment; all these factors and issues may cause under-reporting rather than over-reporting of child abuse. Assessment of childhood adversities using a comprehensive test-retest methodology in young adults has found that self-reported sexual or physical abuse several years later is not as careful as the reports which were made several weeks later (Gilbert et al., 2009).

In order to attempt to determine the effect of depression and childhood abuse separately as explained in the method and result sections, our study recruited 4 distinct groups (non-abused healthy controls, abused healthy controls, non-abused MDD patients, abused MDD patients) rather than simply making a comparison between either MDD patients or healthy controls or abused and non-abused subjects all together.

The comparisons of these 4 groups are discussed in the following section.

4.3.3 Healthy controls without a history of childhood trauma and healthy controls with a history of childhood trauma (CB and CA)

There was no difference in the cortisol or AUC-CAR- between healthy controls without childhood abuse and healthy controls with childhood abuse. Thus, there was no effect of childhood maltreatment on the CAR in healthy controls in our study.

Previous studies have reported conflicting results. An increased CAR was shown in a previous study of healthy young adults who had received low parental care in their childhood compared with healthy young adults who had received high parental care (Engert et al., 2011). Similar to this result, an increased CAR was observed in healthy pregnant women who were sexually abused in their childhood compared with healthy pregnant women and non-sexually abused pregnant women (Bublitz et al., 2012). Considering these studies in detail, Engert et al. (2011) examined 58 subjects between 18 and 30 years of age, consisting of 11 males and 17 females with a history of low parental care, and 8 males and 22 females with high parental care. Parker's questionnaire (1979) was selected to assess childhood maltreatment. All subjects collected saliva by Salivette at awakening and 30 minutes thereafter on 3 non-consecutive weekdays. In comparison to our study, Engert et al. (2011) examined a younger subject group with more males, and used a different method of saliva collection after awakening as explained. They did not control for cycle phase or oral contraceptive use in their analyses, which is in contrast to our study. This is an important consideration because gonadal steroid secretion in females changes during the menstrual cycle and with the usage of oral contraceptives. Gonadal steroids can affect the HPA axis function; thus previous studies have shown that the activation of the stress response and paraventricular nucleus of hypothalamus neurons is higher in

females than males (Seale et al., 2004 ; Viau et al., 2005; Larkin et al.,2010), and that this could be due to the changes in estradiol in the estrous cycle of females (Viau et al.,1991; Rhodes et al., 2004; Iwasaki-Sekino et al., 2009). This is supported by studies investigating patterns of corticosterone secretion, where females present a higher amplitude, frequency and number of corticosteroid pulses compared to males (Seale et al., 2004). However, the mechanism by which gonadal steroid hormones may act to influence the HPA function has not been resolved in these previous studies. Nonetheless, it appears that increased responses to stress are most common in the proestrus phase, when estradiol levels are highest; whilst progesterone may decrease the effects of estradiol on HPA axis function. Taken together, these effects of gonadal steroids on circadian rhythms and the CAR, should be considered as a potential confounder when designing and interpreting studies.

On the other hand, a number of recent reviews have argued that the influence of gender, gonadal steroids or contraceptives use on the CAR is inconsequential (Wust et al., 2004; Fries et al., 2009).

Our abused healthy controls had experienced a variety of types of abuse, differently from the study which focused on parental care only. Another difference lies in the method of assessment of childhood abuse that we selected (Bernstein et al., 2003). Thus, the subjects may not be similar in terms of the nature of childhood abuse they had experienced.

Another study by Bublitz et al. (2012) was conducted on healthy pregnant women; an increased CAR was found in healthy control women with a history of childhood sexual abuse when compared with healthy control non-sexually abused—with other types of abuse—and non-abused healthy pregnant women. 135 participants (childhood sexual abuse n=30, non-sexual childhood abuse n=58, non-abuse

n=47) collected salivary cortisol by the drooling method at awakening time and 30 minutes after awakening for 3 consecutive days at 1-3 time points over the second and third trimester. Subjects were tested with a self-report measure of childhood abuse experiences comprising 15 items from the Adverse Childhood Experiences Scale (Dube et al., 2003). Participants reported on childhood sexual abuse, physical abuse, domestic violence, and physical neglect. In this study, saliva was collected using the same drooling method as our study, but with a lower number of saliva collections post-awakening. Another issue is that this study consisted of pregnant women, where there may be obvious effects of gonadal steroids as explained before. It is unclear whether results in pregnancy are generalizable to results outside of pregnancy, or to males.

It has been suggested that the characteristics of the study population, the type of adversity experiences, and the proneness to psychopathological vulnerability interact to explain whether the CAR is increased or decreased (Engert et al., 2011). In this regard, our subjects consisted of abused subjects with a moderate severity of childhood abuse comprising one or more types of childhood maltreatment (emotional, physical, sexual and neglect-emotional and physical- as explained in the previous chapters).

4.3.4 Healthy controls with a history of childhood trauma and MDD patients with a history of childhood trauma (CA and PA)

The cortisol at 15 and 45 minutes after awakening, and the AUCg-CAR- were higher in abused MDD patients than abused healthy controls. This result is not in agreement with a previous study which found a higher CAR at awakening time and 30 minutes after awakening in healthy controls with a history of childhood neglect compared to depressed patients without a history of childhood neglect (Peng et al., 2014). In detail, they had a total of 28 patients – both inpatients and

outpatients – with a history of childhood neglect (no comorbidity with any other type of childhood abuse) (mean \pm SD age= 28.8 ± 6.28 years; mean \pm SD illness course= 21.68 ± 22.12 months; 15 males and 13 females) and 30 patients – both inpatient and outpatient – without child neglect (There was no comorbidity with any other type of childhood abuse) (mean age= 28.37 ± 8.27 years; mean illness course= 25.48 ± 19.06 months; and , 16 males and 14 females). Their comparison groups were 29 gender and age matched healthy controls without childhood neglect (mean age= 27.87 ± 4.28 years; 15 males and 14 females) and 22 healthy controls with childhood neglect (mean age= 28.37 ± 5.28 years; 12 males and 10 females). Akin to our study the CTQ (Bernstein et al., 1998; 2003) was used to measure early life stress (cut-off score at ≥ 15 for emotional neglect, ≥ 10 for physical neglect). Salivary cortisol was collected at home with Salivettes on two consecutive working days, at awakening and 30 minutes after awakening, representing a lower number of assessments of post-awakening cortisol rather than in our study. In addition, the calculation of the awakening response was not the same as in our study. Instead of using an AUC calculation, HPA axis functioning was characterized as the change between cortisol levels at 30 minutes after awakening and at awakening. The mean value of the consecutive two days was presented as the index of HPA axis functioning. It is of note that they recruited younger subjects with shorter length of illness compared to our subjects. This is important that the age of the subjects could have influenced the CAR, since older age is associated with a decreased CAR (Kudielka et al., 2003; Rhebergen et al., 2015).

Of note, Peng et al (2014) examined both outpatient and inpatient subjects; this too might have affected the CAR, as previous studies have found an increased CAR in healthy subjects with depressive symptoms and in acute MDD outpatients

compared to a blunted CAR in inpatients (Pruessner et al., 2003; Huber et al., 2006). The number of saliva assessments differed between our study with Peng et al. (2014), and it could have affected the results; Of note, appropriate results of the CAR were observed in the previous studies in which the CAR were assessed on minimum of 2 days rather than one day, representing that we could decrease the biases of our results, by rising the number of assessment to 2 consecutive days (Wust et al., 2000; Hucklebridge et al., 2005). Another important factor is that Peng et al. (2014) focused on neglect in childhood, whereas our study consisted of a wider range of abuse types or of multi-abuse comorbidity. Thus our results might be a consequence of the cumulative effect of several types of abuse rather than one type of abuse.

However, In association with this, previous studies which investigated ELS either in MDD patients or healthy controls argued that the alteration of the CAR, both decreased and increased, could be observed (Earls et al., 1997; Stein et al., 1997; Heim et al., 2001; Gunnar et al., 2001; Roy et al., 2002; Weissbecker et al., 2006; Power et al., 2012, Peng et al., 2014).

4.3.5 Healthy controls without a history of childhood trauma and MDD patients without a history of childhood trauma (CB and PB)

Our study found no effect of depression on salivary cortisol or AUC-CAR- in non-abused patients compared with non-abused controls. This result is not consistent with the results of numerous studies that have found a hyperactivity of the HPA axis in MDD patients compared with controls (Gold et al., 1985; Holsboer et al., 1985; Sacher et al., 1973; Bhagwagar et al., 2005; Cowen et al., 2010; Vreeburg et al., 2009). Of note, among all the previous studies, there were some confounding factors which could affect the results. For example, the alteration of

the CAR – whether increased or decreased – could be associated with the characteristics of the subjects (Bauer et al., 2002, Weber-Hamann et al., 2002, O'Brien et al., 2004; Peeters et al., 2004; Juruena et al., 2006; Khnor et al., 2010) which could play an important role in incontinuity of the results.

Another confounding factor about previous studies showing HPA axis hyperactivity is that, they did not usually assess the history of probable childhood abuse, which our results suggest to have an effect on HPA axis activity in depression, and could possibly explain the differences in those studies compared to our findings. In particular, because of the high unreported experience of ELS among MDD patients and healthy controls, numerous previous studies have not been able to separate out the effect of ELS and the effect of MDD. It suggests that previous findings about the neurobiology of depression might be confounded due to the lack of clear reported of childhood abuse.

Considering each of these studies, Bhagwagar et al. (2005) conducted a study on 20 patients without medication (mean age= 43.6 ± 11.0 years; 10 male and 10 female) recruited from primary care sources with a Hamilton score of 19.8; and 40 healthy controls (mean age= 40.5 ± 13.6 years; 19 male and 21 female). Saliva collections were obtained by the participants at home, using Salivettes, at awaking time and at 15 minutes intervals for the next hour. The time of the menstrual cycle in female subjects was not controlled. Two controls and three patients were on oral contraceptives at the time of the study. Increased salivary cortisol was found in MDD patients compared to healthy controls; and the authors suggested that this was independent of age, gender, and hours of sleep, time of awakening and severity of depression.

It is noteworthy to consider that in both Bhagwagar et al's study and ours, there were a number of issues which could have affected the results and led to biases.

First of all, it has been well documented that delays of 5-15 minutes between awakening and the beginning of saliva collecting should be considered in the assessment of the CAR (Smyth et al., 2013). In our studies, the actual timing of each saliva collection was assessed by using self-reported times, which might not be the exact time of collection in all cases, even though both studies asked subjects to report the exact time of collecting. Second, awakening time in controls and patients in both studies differed; this could confound results as MDD subjects usually wake up earlier than controls (Edwards et al., 2001). Bedtime and total sleep duration were not considered in both studies, which could affect the result; as a number of studies could show a clear effect of awakening time with subjects waking up early having a higher CAR than subjects waking up late (Edwards et al., 2001; Kudielka et al., 2003; Aubry et al., 2010).

Bhagwagar et al's results are not similar with our finding and this could be related to a number of issues. The most important factor could be the characteristics of subjects of the studies, in that they did not consider a history of childhood abuse in their subjects which could have affected their results; of note, this is an important issue because if they could consider this issue, the results might be consistent. Another issue was that they recruited un-medicated MDD patients from primary care, whereas our study was conducted in inpatients and outpatients who were receiving several types of antidepressant. The use of antidepressant medication is a potential confounding factor; as previous studies have shown a flattened CAR in association with tricyclic antidepressant (TCA) use, and higher evening cortisol levels and decreased cortisol suppression after dexamethasone ingestion with the use of SSRIs (Manthey et al., 2011; Khnor et al., 2012). In addition, in regards to the study by Kunz et al. (2004), the day of saliva collection i.e. working or non-working day, or weekends versus weekdays,

is a crucial factor that can affect results as higher CAR on weekdays than weekends and working days than non-working days has been found (Kunz et al., 2004; Schlotz et al., 2004). Although, the majority of subjects in Bhagwagar et al. (2005) did not collect saliva on working days, making it unlikely that this was a factor that could affect their results, it is clear that this confounding factor should be considered in their future studies.

As mentioned before, another issue is that the lack of control for menstrual cycle phase and use of contraceptives could be a potential confounding factor as the CAR may be increased during ovulation (Bouma et al., 2009; Wolfram et al., 2011) which means, if their subjects collected saliva during ovulation, Bhagwagar's results may be influenced, as Wolfram et al. (2011) showed the higher CAR during ovulation compared to the menses, the follicular phase and the luteal phase in healthy controls. However, to our knowledge, the effect of ovulation on CAR specifically in MDD patients is unclear.

In sum, our study did not show a significant difference in the CAR between non-abused healthy controls and non-abused MDD patients in contrast to previous studies.

4.3.6 Healthy controls without a history of childhood trauma and MDD patients with a history of childhood trauma (CB and PA)

A higher cortisol and AUC-CAR- at all time points except at time of awakening was observed, which raise the assertion that the effect of early life stress and depression may have an additive or cumulative effect on basal cortisol and the abnormal responsivity of the HPA axis shown by the heightened CAR.

4.3.7 MDD patients without a history of childhood trauma and MDD patients with a history of childhood trauma (PB and PA)

Although an influence of ELS on the CAR in healthy controls was not observed in our study, the comparison between non-abused MDD patients and abused MDD patients did show an effect of ELS on the cortisol at 15 and 45 minutes after awakening and an increase in the AUC-CAR. This result is consistent with a previous study, showing increased salivary cortisol at awakening time and 30 minutes after awakening in MDD patients with history of childhood neglect compared with MDD patients without a history of childhood neglect as explained before (Peng et al., 2014). To our knowledge, there is no study that has looked at the effect of other types of ELS than neglect in MDD and its relation with CAR. In association with this, previous studies have shown that adult women with a history of childhood sexual or physical abuse exhibit increased neuroendocrine and autonomic reply to psychosocial laboratory stress, in particular, those with MDD (Heim et al., 2001). Other changes demonstrated in subjects with ELS experiences have included glucocorticoid resistance and increased central corticotropin-releasing hormone activity (Heim et al., 2012).

In association with this, the investigation about MDD and the CAR in previous research, shows there is a strong association between them, presenting the important character of the CAR in MDD patients. It was argued that there is a relation between the CAR and several risk factors of depression. It was suggested that the CAR is a predictor of stressors to come such as increasing the CAR on working days. As explained before, MDD itself has been associated with both increased and lowered or blunted CAR; even attempts have been made in investigating whether CAR may serve to anticipate depression onset (Dedovic et al., 2015).

However, our study did not separate out the different types of abuse and we cannot conclude which particular types of abuse played the most important role in the changes to the cortisol or AUC-CAR- in the abused MDD groups.

4.3.8 All the groups

In sum, MDD patients who suffered from childhood abuse presented the highest CAR among all the groups. Our study showed that the total cortisol released in the period after awakening AUCg-CAR- increased when patients were abused in their childhood.

4.4 Clinical results

Although a previous study argued that the type of treatment did not seem to have an influence on cortisol (McKay et al., 2010), an association between antidepressants and cortisol was observed in a previous study by Manthey et al. (2011). This was conducted on 1526 subjects who were using a serotonin reuptake inhibitor (SSRI) (n=309), TCA (n=49) or other type of antidepressants (n=100), or who were non-users of medication (n=1068). All subjects had a current or past diagnosis of anxiety and/or depression. As compared to non-users, TCA users had a flattened CAR; SSRI users had higher evening cortisol levels and they showed decreased cortisol suppression after receiving dexamethasone ingestion. By findings of their study they suggested that different subtypes of antidepressants were associated with distinct alterations of the HPA axis. TCA users, who presented a flattened CAR, showed the strongest alterations of salivary cortisol. Another study by Ruhe et al. (2015) conducted on MDD patients without medication and healthy controls investigated the change from the baseline CAR after 2 weeks of Paroxetine treatment during 12 weeks. Although the study did not show a significant difference in the CAR between MDD

patients and healthy controls at baseline, they observed a reduced CAR in MDD-patients after 12 weeks of treatment; especially when patients achieved remission.

Thus, it is important to consider that the MDD patients in our study were using several types of antidepressant, which might have affected the CAR. We were not able to compare medicated to un-medicated subjects due to the small numbers who were without medication, nor did we have sufficient power to compare differential effects of the type of medication patients were taking. However, we did not observe a strong and significant correlation between the usage of antidepressant and cortisol values or AUC-CAR.

Of note, investigating the CAR in remitted patients could be helpful in understanding the effect of previous depressive episodes on the HPA axis. In line with this, Aubry et al. (2010) conducted a study on 38 remitted MDD patients (age =24-66 years; 11 men and 27 women) and 52 healthy controls (age=24-63; 18 men and 34 women); all patients were without medication at least for 3 months and they had experienced at least 3 previous depressive episodes. Saliva was collected at home, either at the weekend or on a weekday, at 0, 15, 30, 45 and 60 minutes after awakening. The results supported the hypothesis that remitted MDD patients continue to show an increased CAR in compared with controls. In line with this, in previous studies, it has been argued that in the long-term course of depression, the HPA axis abnormality increases in parallel with the number of previous episodes (Aubry et al., 2010).

Our study found MDD patients who had experienced their first episode later in life showed a higher individual cortisol at 15 minutes after awakening; although this result could be explained by gender of the patients, as we recruited more females rather than males in our patients group and the CAR is higher in female compared

to male(Dedovic., 2015). In line with this, we should consider about the age of our subjects; as older age seems to be related to lower CAR, rather than higher CAR (Kudielka et al., 2003); although it does not seem our finding could be confounded by the age of our patients as we recruited older patients rather than younger ones.

4.4.1 Type of abuse and severity of depression

Childhood abuse is an important environmental risk factor which can increase the risk of developing depression (Heim et al., 2008). Hoven et al. (2015) showed that emotional neglect, physical and sexual abuse were associated with an increased risk of first onset and relapse of MDD. They showed that a higher severity of depressive symptoms at baseline and the presence of a prior lifetime diagnosis of a depressive and/or anxiety disorder, mediated these associations. This study argued that emotional neglect was the most important predictor of first onset and recurrence of any depressive or comorbid disorder among all types of abuse.

The results of our study showed a positive correlation between the severity of depression in terms of Hamilton score (HAM-D 17, 21) and sexual and emotional abuse, and are thus supportive of the findings of Hoven et al (2015).

4.5 Self-rated questionnaires

4.5.1 Work and Social Adjustment Scale (WSAS)

The level of functioning of MDD patients is not highlighted in standard symptomatic measures of depression severity, such as HAMD-17. That is the reason that DSM-V has introduced a new measure of functioning (e.g. the WHO Disability Assessment Schedule) to provide a more comprehensive assessment of functioning in patients with psychiatric disorders (Lin et al., 2015). Although

symptoms recover within several weeks after beginning of treatment, functioning does not improve as quickly (Lenderking et al., 1999).

In our study, abused and non-abused MDD patient groups both presented higher scores on the WSAS compared with the healthy control groups, supporting the assertion that MDD affects the everyday tasks of the subjects. We could not observe any effect of ELS on the score of WSAS.

There was a positive correlation between CAR and the WSAS, signifying that when level of function decreases, the cortisol is higher. It should be noted that in healthy controls, physical good health and better function is related to higher CAR. Our result is a correlation between CAR and the score of both healthy controls and patients; thus, we could not specify that this higher CAR correlated with higher level of function is the impact of MDD or general good health of our controls.

4.5.2 Zung self-rating anxiety scale and anxiety index (Zung)

Moffitt et al. (2007) in a cohort study revealed that there is a strong association between GAD and MDD; their study investigated the cumulative and sequential comorbidity of these disorders. The sequential overlap of GAD and MDD is considered to involve an initial onset of anxiety, followed by development of MDD. Longitudinal studies have argued that childhood anxiety predicts later MDD.

Carr et al. (2013) in a systematic review argued that ELS is associated with anxiety disorders. In particular, physical abuse is related to PTSD and the severity of anxiety disorder. Sexual abuse is related to PTSD, panic disorder, agoraphobia, and obsessive-compulsive disorder. Emotional abuse is associated with social phobia combined with substance abuse. Emotional neglect is generally related to the severity of psychopathology.

In our study, MDD patients compared to healthy controls and abused subjects compared to non-abused subjects, had higher scores, which confirms that these subjects suffered from more level of anxiety.

The positive correlation between CAR and the degree of anxiety supports the argument that anxiety is associated with higher CAR. This finding is consistent with the study conducted by Vreeburg et al. (2010) which showed current anxiety disorder was related to higher CAR. In details, their findings were based on patients with panic disorder with agoraphobia and anxious patients with comorbid depressive disorder. They also could show trend toward higher morning cortisol for remitted anxiety patients.

4.5.3 Medical Outcomes Survey Short Form 36 (SF-36)

Childhood abuse may have a strong effect on general health, with evidence of long terms effects on health outcomes in adulthood (Font et al., 2015). In consistent with this, in our study, non-abused subjects and healthy controls had higher SF-36 scores, confirming that these subjects had higher levels of general well-being.

The correlation between the CAR and SF-36 was negative, showing that with increasing general health, the CAR was lower , which was not in agreement with the study conducted by Kudielka et al.(2003), in which the higher CAR was associated with physical health. Of note, we made the correlation among all the subjects which consisted of MDD and controls with and without a history of abuse; thus, it could be the reason of this incontinency between our results with theirs.

4.6 Strengths and Limitation

4.6.1 The first study

To our knowledge, there are no studies that have investigated the modulatory effects of the BDNF val66met polymorphism on grey matter amygdala volume in MDD; thus, the novelty of the work is an important strength of our study.

Nevertheless, there are a number of limitations within the first study. First, our study was restricted to the analysis of one priori region of interest, the amygdala, although as noted earlier, the study was a part of a wider study in which other regions of the brain were investigated (see Appendix A).

The lack of data on the history of early life stress in the subjects could be one of the important factors, as there is evidence that stress could lead to brain atrophy by modifying BDNF levels (Gerritsen et al., 2012); and reduced grey-matter volume in the amygdala and hippocampus was observed in healthy subjects carrying the met allele with a history of greater stressful events (Gatt et al., 2009) ; thus we could investigate whether this polymorphism modifies the effect of early life stress on grey matter amygdala volume. Another restriction could be that the most of the subjects with MDD were taking antidepressant medications, which could have an effect on neural volumes. Medication and treatment influences on amygdala volume have not been well documented in previous studies; thus, we cannot exclude an effect of medication on our results.

Another factor which should be considered is that in our study we classified met-homozygous and heterozygous in one group-named met carrier, due to small population of met/met homozygote, therefore, there is a possibility that we miss the real effects of met homozygotes on amygdala volume.

4.6.2 The second study

In terms of strengths, we considered and attempted to control for numerous confounders to maximize the validity of the results. For example, previous studies showed that the day of the week is important as the CAR is higher when measured on a weekday than on a weekend (Edward et al., 2003; Kudielka et al., 2003). Because of this we controlled the day of the collections of the subjects and we measured the CAR on weekdays. We considered smoking, alcohol and drug consumption in our subjects and we decreased the confounding effects of these factors on our results by not recruiting heavy smokers or alcohol and drug dependent subjects.

Our study did not include patients with an identified organic aetiology to their psychiatric diagnosis. Moreover, we did not include subjects with major physical illnesses, or medication use, in particular corticosteroids that might affect the HPA axis. We excluded pregnant or lactating women. For healthy controls we did not include those with a history of psychiatric illness in their first degree relatives. All of these factors will have minimized the possibility that factors other than depression or childhood trauma could have affected the observed changes in the CAR between groups.

It is noteworthy that there is no previous study which has demonstrated the additive effect of MDD and early life stress together on CAR as our study has done, emphasizing the novel nature of these findings. There have been a number of studies in which the effect of early life stress or the influence of MDD has been investigated, but to our knowledge, there have been no studies that have investigated the effect of early life stress and MDD in four groups of healthy controls and MDD patients, and using multiple time points to evaluate the CAR. Our study made a comparison between abused MDD and non-abused MDD, and

showed a differential pattern of HPA dysfunction in MDD patients on the presence or absence of ELS, a novel finding.

Against these strengths, we need to be weighed some limitations. Although we did control for many potential confounding factors, one of the limitations of our study is the presence of some other important factors we were not able to control. In detail, more reliable and true investigation of the CAR have been observed in the previous studies in which the CAR was investigated on a minimum of 2 days. This may be because collecting the cortisol for more than one day can minimize the effect of random factors that might affect the CAR if collected on just one day (Wust et al., 2000; Hucklebridge et al., 2005; Deodovic et al., 2015).

Gender could be an important factor as females have been found to have a higher CAR than males in previous studies (Pruessner et al., 1997; Wust et al., 2000). Our subjects were not fully matched for gender. There were significant differences between groups in terms of gender (Male and Female, CB = 19 and 24; CA = 14 and 12; PB = 5 and 10; PA=5 and 23) as we found a higher CAR in PA compared to PB, and in PA compared to CA, and the PA group had the highest number of females, it is possible that our result could have been affected by the higher number of females in PA compared to PB or CA.

Although older age has been associated with a lower CAR and we had older MDD patients than healthy controls, it was unlikely to have affected our results as the CAR was higher in MDD patients than healthy controls (Kudielka et al., 2003).

The time of awakening of the MDD patients was not standardized and could be a confounding factor as earlier awakening time is associated with a higher CAR (Markopoulou et al., 2008; Deodovic et al., 2015).

Another issue which should be considered is that the groups were not divided in regards to the sub-type of abuse: all subtypes of abuse were considered together.

Therefore, it is not clear whether it was one or more particular types of abuse, or in fact the cumulative effect of several types of abuse, which were the important factors mediating the effects of childhood trauma on the CAR.

The use of antidepressants by the MDD patients is another limitation of our study, as antidepressant use might affect the HPA axis and the CAR as explained before (Manthey et al., 2011; Ruhe et al., 2015). It would have been ideal if patients were all using the same type of antidepressant or were un-medicated rather than taking several types of antidepressant.

The assessment of early life stress was restricted to a self-report questionnaire measure of childhood experiences, whereas formal interview may give more comprehensive evaluation of the objective level of early life stress (Suzuki et al., 2014). However, this assessment using the CTQ is a well-validated and widely used approach used in the literature. Finally, there are several potential unmeasured confounders that might be associated with cortisol collection, such as sleep quality, on which we were not able to gather appropriate information from our subjects.

4.7 Future studies

4.7.1 The first study

One of the important factor which should be considered is the possible epistasis of genes. Future studies need to focus on the interaction of BDNF and the other genes such as 5 Serotonin-Transporter-linked Polymorphic Region (5HTTLPR) (Wang et al., 2012; Grabe et al., 2012), because based on biological interactions between the serotonergic system and BDNF, BDNF is a reasonable candidate for a gene-gene-environment interaction moderating the interaction between the s/l- promoter polymorphism of the serotonin transporter (5-HTTLPR) and childhood abuse.

Another gene which might be a new candidate in MDD is Catechol-O-methyltransferase (COMT) which is a methylation enzyme engaged in the degradation of dopamine and noradrenaline by catalyzing the transfer of a methyl group from S-adenosylmethionine. It is noteworthy to investigate about the interaction of BDNF and COMT as there is evidence about val158met COMT polymorphism in association with MDD (Cao et al., 2014); an effect of val108/158met COMT on brain morphology (e.g. smaller caudate volume) in patients with first episode and treatment naïve MDD compared to healthy subjects (Watanabe et al., 2015); and epistatic interaction of BDNF and COMT on the frontostriatal system which is an important area to play a role in cognition and emotion (Wang et al., 2015), were observed before.

In our study there were no measures of possible environmental factors that could influence the expression of the genetic vulnerability into reduced amygdala volume. These could include recent as well as childhood stresses. Thus, in future studies, both environmental and biological factors should be included as there seems to be a link between these factors such as stress and BDNF (Gatt et al., 2009). Furthermore, the effects of treatment, as well as the type of treatment used (such as antidepressant class or CBT) should be considered; as these treatments could affect the results (Choi et al., 2006), and in line with this, the effects of treatment on brain structure, and whether there are differential effects of such treatments on brain structure depending on genotype, needs to be considered and accounted for in future studies. Furthermore, BDNF may be implicated not only in depression but in other psychiatric disorders (Green et al., 2006), and it may affect the other parts of brain such as the anterior cingulate cortex (Gerritsen

et al., 2012) which is thought also to play a crucial role in MDD and in the response to treatment.

4.7.2 The second study

The work to date shows that CAR is immensely heritable which suggests that to a certain degree, it is a trait-like feature. As it is also highly affected by environmental and situational factors, it can serve as an index of one's overall vulnerability to depression as it is associated with stressful experiences.

Findings from the longitudinal studies suggest that increased CAR being linked with MDD onset and recurrence. Focusing on the link between stress and depression, it is not only the number of major stressful events experienced but also the type of stress and one's perceived levels of stress and coping abilities that are of importance. In association with this, CAR could be particularly relevant when it comes to recurrence of depression, given that the onset of the first depressive episode is often linked to experience of a major stressful event, whereas the following episodes could be triggered by more minor events, due to stress sensitization.

Previous studies suggest that in the healthy controls, increased CAR is associated with being able to meet specific demands of the upcoming day (e.g. workday vs weekend), and decrease of stress on that certain day, and therefore has been linked to coping (Deodovic et al., 2014). It is noteworthy to wonder that this association may become dysregulated once coping employed is ineffective in removing feeling of stress during time. In that case, heightened CAR (and possibly inflexible or stiff CAR) may switch from signaling coping to signaling prediction of stress of the day after. It has been argued that it is likely that at a specific threshold, persistent increased CAR may be downregulated, and become blunted. This could be the reason for the observation of heightened CAR

and others blunted CAR in MDD patients. These factors can be investigated in future studies.

In line with this, future research should focus on differential effects of individual types of early life stress as the effect of specific types of abuse have not been well documented either in the current study or in previous studies (Heim et al., 2000; 2010). It has been argued that poor adult health is associated with childhood physical, emotional, and sexual abuse (Font et al., 2015). Therefore, investigating the influence of specific type of early life stress on health outcomes in adulthood would be an important and interesting area to develop this work further.

In order to clarify that whether the CAR abnormalities anticipate the onset of depression, are present in the first episode, whether they are associated with the number of recurrences or the total duration of illness episodes, future research would also need to measure the endocrine factors during earlier stages of the illness, ideally in the first episode and in subjects that are not taking medications. Increased salivary cortisol might be a predictive biomarker of MDD in both young people and adults (Goodyer et al., 2000; Harris et al., 2000); from this point of view, salivary cortisol secretion has the characteristics of an endophenotype of MDD and it might be a reasonable marker for genetic analysis. This is likely to be a fruitful area in future research (Cowen et al., 2010). However, the contribution of genetic polymorphisms in certain genes such as those encoding the corticosteroid receptors has not been well documented. It has been argued that carriers of the short allele of the serotonin transporter exhibit an increased salivary awakening cortisol but the results have not been consistent (Mannie et al., 2008).

In sum, the association between CAR and vulnerability to depression is important but complex; and we found an additive association between MDD and early life stress on the CAR. In line with this, our findings suggest that MDD patients with a history of early life stress could be considered as a specific group which is more sensitive to show the abnormality of HPA axis and it might argue that early intervention, supportive care, and specific or combined treatment could be useful to prevent the next depressive episode in these subjects. Further research with comprehensive methods could bring more insight into other association between CAR, MDD and ELS.

4.8 Conclusion

4.8.1 The first study

Inconsistent results had been found about the role of BDNF in the pathogenesis of MDD and its effects on grey matter of amygdala volume in previous research. Evidence from neuroimaging studies about the important effects of the BDNF val66met polymorphism on amygdala volume in healthy controls (Montag et al., 2009), and the association of the val66met polymorphism with reduced hippocampus volume – as a part of limbic system – in MDD patients compared to controls (Frodo et al., 2007), raised the assumption that the BDNF val66met polymorphism may have a considerable influence on the amygdala volume in MDD patients. Supporting this argument, decreased amygdala volume in unmediated patients (Hamilton et al., 2008) and alteration of amygdala volume in first episode MDD patients (Frodo et al., 2002) had also been found in previous studies.

However, our study did not find a clear effect of met allele on the amygdala volume either in healthy controls or MDD patients.

4.8.2 The second study

The study attempted to find a link between abnormalities in the CAR and early life stress in MDD patients in order to identify a biomarker of MDD, which could be important either in prevention or treatment of MDD.

In healthy controls, the study found no effect of a history of childhood abuse on the CAR.

In MDD, disregarding the presence or absence of childhood abuse, the CAR was higher in MDD patients compared to healthy controls. Both the AUC_G and the AUC_I were higher, suggesting higher total secretion of cortisol and a hyperactivity of the HPA axis in MDD patients.

However, this result obscured a significant difference in MDD patients in association with their history of childhood abuse. The CAR was higher in those MDD patients with a history of abuse compared to those without, supporting the argument that early life stress may play a major role in the aetiology of HPA axis hyperactivity in MDD. Consistent with this result, the CAR in non-abused MDD patients was not increased compared with non-abused healthy controls.

A major suggestion of the study is that many of the previous studies that have found HPA axis hyperactivity in MDD have not assessed for the presence of childhood abuse. It may be that MDD in patients with a history of childhood trauma has a certain aetiology that involves HPA axis hyperactivity, whereas MDD in those without such a history is not associated with the same, or indeed any, HPA axis changes.

These results could support the argument that the effect of childhood abuse and MDD on the CAR are additive. On the other hand, the CAR in abused MDD patients were higher than all three other groups, which could support the idea that the combined effect of MDD and early life stress on the CAR can cause HPA

axis hyperactivity. It is noteworthy that this is a novel finding in the literature. Our study did not observe any change in the CAR either in abused healthy controls compared with non-abused healthy controls or non-abused MDD patients compared with non-abused healthy controls, thus showing that there is no influence of early life stress on the CAR without the additional effect of MDD. Similarly, no clear effect of MDD on the CAR was seen without the presence of a history of early life stress. In contrast, abused MDD patients compared with abused healthy controls showed higher CAR and total secretion of cortisol; abused MDD patients compared with non-abused healthy controls demonstrated higher CAR and total secretion of cortisol and abnormal HPA response; and the comparison of abused MDD patients and non-abused MDD patients showed an important effect of early life stress in MDD.

Our study found that the CAR increases when the severity of emotional, physical and sexual abuse increases. We also showed that there is an association between anxiety and the CAR, and that anxiety levels are higher in abused MDD patients. The result on the SF-36 questionnaire provide further confirmation of the marked effects of MDD on all aspects of patients' lives and functioning.

In terms of clinical outcomes, we showed that a later onset of the first episode of MDD is associated with an increase in cortisol levels at 15 minutes after awakening in MDD patients, although this association could also be confounded by other clinical factors such as the number of previous episodes and duration of current illness.

4.8.3 Overall Conclusion

The two studies outlined in this thesis focused on several of the major areas where progress is being made in the attempt to solve the riddle of depression,

i.e. genetic, neuroimaging and endocrinology research. Although we found no modulatory effect of val66met BDNF polymorphism on amygdala volume in MDD patients, we showed that the effect of early life stress and MDD are additive in that the CAR is most increased in those who are both depressed and have a history of early life stress. This finding supports the argument that an association between MDD and the CAR is only observed in those with a history of abuse. This is a novel finding, and of importance in trying to unravel the aetiology of HPA axis dysfunction in MDD.

However, MDD and child abuse remain global concerns; many children and adults are continuing to suffer from the damaging consequence of MDD and early life stress. It is hoped that one of the outcomes of the work in this thesis is to help lighten the darkness around MDD and child abuse.

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Appendix A. The First Study

Modulatory effects of brain-derived neurotrophic factor Val66Met polymorphism on prefrontal regions in major depressive disorder

Legge RM*, Sendi S*, Cole JH, Cohen-Woods S, Costafreda SG, Simmons A, Farmer AE, Aitchison KJ, McGuffin P, Fu CH.

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Abstract

BACKGROUND:

Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism contributes to the development of depression (major depressive disorder, MDD), but it is unclear whether neural effects observed in healthy individuals are sustained in MDD.

AIMS:

To investigate BDNF Val66Met effects on key regions in MDD neurocircuitry: amygdala, anterior cingulate, middle frontal and orbitofrontal regions.

METHOD:

Magnetic resonance imaging scans were acquired in 79 persons with MDD (mean age 49 years) and 74 healthy volunteers (mean age 50 years). Effects on surface area and cortical thickness were examined with multiple comparison correction.

RESULTS:

Subjects who were Met allele carriers showed reduced caudal middle frontal thickness in both study groups. Significant interaction effects were found in the anterior cingulate and rostral middle frontal regions, in which participants in the MDD group who were Met Carriers showed the greatest reduction in surface area.

CONCLUSIONS:

Modulatory effects of the BDNF Val66Met polymorphism on distinct subregions in the prefrontal cortex in MDD support the neurotrophin model of depression.

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Appendix B. The Second Study

HORMONE MEASUREMENT

Saliva measurements of hormonal concentration

Saliva cortisol concentrations were determined using the chemiluminescence assay of 'Immulin'- DPC's automated Immunoassay analyzer. (www.diagnostic.siemens.com).

- a. The cortisol concentrations of unknown saliva specimens were read off a calibration graph constructed from 10 cortisol standards in saline- concn 0- 160 nmoles/L.
- b. The volume of the specimen taken for analysis was increased by 40UI.

Multicalc v 2.65 (part no 1224-310; www.perkinelmer.co.uk) was used to plot the calibration graph and for immunoassay data processing.

The method correlated well with an in-house and previous published study (Jurueña et al., 2006) TR-FIA ($r=0.94$, $y=0.004+1.08 x$, $N=41$), had analytical sensitivity of 0.2 nm/l, mean % recovery of cortisol of 106.1 (range 5 to 65 nm/l).

And inter/intra-assay precision (% CV) of less than 10% (range 5 to 25 nm/l).

The linearity upon dilution test (parallelism) resulted in a straight line ($r=0.99$, $y=0.144+1.014 x$). The calibration graph was highly reproducible ($n=11$ assays) with slope (mean \pm sem) of 0.197 ± 0.004 .

The percentage cross reactivity of the antiserum with cortisol was 0.35%.

The short and long term performance of the assay was monitored with

- a. Biorad commercial control sera (www.bio-rad.com)
- b. Human saliva pools and

- c. Participation in external QC scheme for saliva steroids organized by IBL
(www.ibl-hamburg.com)

Saliva specimens collected at each time point were defrosted, mixed and after centrifugation at 3500 rpm at room temperature, were treated as explained below. In brief, using the Genesis 100 Robotic Sample processor (Tecan UK, Theale, Reading, UK), 50 μ L of the test saliva or standard was boosted to the wells of microtitration strips. This was followed by 50 μ L of a solution of the enzyme labelled hormone and 50 μ L of the hormone antibody. Both of these reagents were boosted using an electronic Eppendorf Repette.

After an incubation of 4hrs at room temperature, the incubation solution was abandoned and the wells were washed 4 times with 250 μ L of wash out buffer and 50 μ L of chemiluminescence reagent. The luminescence of the bound fraction was measured in a Berthold Luminometer (MPLI, Berthold Detection Systems, and Pforzheim, Germany) which was linked to MicroWin 2000 Version 4 (Microtek, Laborsysteme, Ovoroth, Germany) for immunoassay data processing.

We used the Detailed Instructions for Saliva Collection of previous studies, although we explained to each subject in detail what they need to do.

Detailed Instructions for Saliva Collection

We ask you to collect your saliva on 4 consecutive days or if not then as close to each other as possible. You can decide which days.

Choose the days when you are in good health and have no heavy social commitments i.e. late evenings, parties, social gatherings, entertainment and so on.

We will provide you with 4 small plastic bags. These will be labelled as DAY 1, DAY 2, DAY 3, and DAY 4. They will contain the tubes you need to collect your specimens in. All tubes are labelled on the outside and numbered at the top.

Each day we ask you to collect your saliva immediately upon waking up (whichever time that is) and then at +15 mins, +30 mins, +45 mins, +60 mins, +90 mins and before your lunch. We also ask you to collect an evening specimen at 10 pm, a total of 8 specimens.

We will give you a form to fill in. Please complete this every time you collect your specimen inserting the date, time, you woke up and collected the specimens or telling us something about the events that took place the hour before you collected the specimens.

DAY 1,2,3,4

Morning specimens

Wake up between 6 and 9:30 am and take the tubes from the bag labelled DAY 1, 2, 3 or 4 according to the days you are collecting.

Do not brush your teeth and do not have breakfast or any other drinks. You can clear your throat before starting to collect specimens.

Sit by your bed or choose another quiet part of your bedroom and make a note on the collection form of the exact time you woke up e.g. 7.15 am. Start collecting your specimens in the tubes provided closing them firmly after each collection and noting the time on the collection form. Remember you need to fill each tube with clear saliva at least up to the 1 ml mark and above.

Take tube No 1 (labelled immediately on wake up) and collect your saliva.

15 minutes after waking up take tube no 2 and collect your saliva. Note the time on the collection form.

30 minutes after waking up take tube no 3 and repeat the saliva collection.

45 minutes after waking up take tube no 4 repeat the saliva collection.

60 minutes after waking up take tube no 5 repeat the saliva collection.

90 minutes after waking up take tube no 6 repeat the saliva collection.

Return your tubes into the plastic bag labelled DAY 1, 2, 3 or 4 and store in the freezer/leave your room.

Throw away your plastic straws once you have finished using them.

You can have breakfast now and continue with your other normal daily activities.

Before your lunch take tube no 7 and repeat the saliva collection.

Evening specimen

At 10 pm choose a quiet part to stay and preferably sitting down and take the tube labelled 10 pm and collect your saliva. Make a note on the collection form of the exact time e.g. 10.15 pm that you collected the specimen.

Return the tube to the bag labelled DAY 1 and leave in the freezer/your room.

Remember it is important that in the HOUR before giving the specimen you should try and avoid the following as these will affect the hormone measurements.

Eating or drinking anything- if you do have something accidentally you must make a note of it.

Demanding social activities i.e. late night parties/entertainments or any hassles e.g. arguments with friends/relatives, difficult conversations. If you cannot then make a note on the collection sheet.

You should make a note if you are in any kind of pain (headache, migraine, toothache, backache) or feeling sick for some reason.

IMPORTANT POINTS TO HELP YOU COLLECT ENOUGH SALIVA WITH THE “STRAW AND TUBE METHOD”

Collect enough saliva in your mouth.

Take the appropriate tube and plastic straw out of the labelled bag, remove the cap and place one end of the straw in your mouth and the other end low down, near the bottom of the collection tube.

Slowly pass the saliva/spit through the straw to the tube- do not blow otherwise this will cause frothing. Tap gently the bottom of the tube on a table top a few times – this helps a clear layer of saliva collect at the bottom of the tube and reduces “frothing”.

Move the end of the straw above the surface of the collected saliva in the tube and repeat the process until you have collected enough – well above the 1 ml mark on the tube.

Close the tube tightly, put it in the appropriate bag and leave it in the freezer/your room.

Remember

Morning specimens- these should be collected before you brush your teeth and before you have any breakfast or other drinks.

Evening specimens- Have your evening meal early. Do not have anything to drink or eat or chew gum or smoke about 1 hour before giving the specimen.

To make a note on the collection forms the time you collect each specimen and also if you experience any hassles (e.g. difficult conversations, arguments with friends/relatives) pain of any kind e.g. headache, migraine, toothache.

Post all your specimens to the National Affective Disorders Unit. We shall provide you with an envelope addressed to the unit.

STUDY

Subject Code= Patient/Control

Name Age

Date

PLEASE DO NOT BRUSH YOUR TEETH EAT OR DRINK ANYTHING FOR AT LEAST ONE HOUR BEFORE THE COLLECTION. IF YOU DO HAVE TO DRINK WATER PLEASE DO IT IMMEDIATELY AFTER THE COLLECTION OF THE SAMPLES

Tube No	Sample	Time given	Comment	Lab Code
1	Immediately on waking up		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	
2	Waking+15 minutes		Where were you? How were you feeling? What were you doing before the collection of the samples? Did you accidentally eat or drink?	

3	Waking+30 minutes	<p>Where were you? How were you feeling?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you accidentally eat or drink?</p>
4	Waking+45 minutes	<p>Where were you? How were you feeling?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you accidentally eat or drink?</p>
5	Waking+60 minutes	<p>Where were you? How were you feeling?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you accidentally eat or drink?</p>
6	Waking+90 minutes	<p>Where were you? How were you feeling?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you accidentally eat or drink?</p>
7	Midday	<p>Where were you? How were you feeling?</p> <p>What were you doing before the collection of the samples?</p> <p>Did you accidentally eat or drink?</p>
8	10 pm	<p>Where were you? How were you feeling?</p> <p>What were you doing?</p>

Did you accidentally eat or drink?

What were you doing before the collection of the samples?

Did you eat or drink?